(19) Europäisches Patentamt
European Patent Office
Office européen dee brevets

(11) Publication number: **0 344 029**
A1

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 89401194.9

(22) Dete of filing: 26.04.89

(51) Int. Cl.[4]: C 12 N 5/00
C 12 N 15/00, A 01 H 1/00

(30) Priority: 28.04.88 GB 8810120

(43) Dete of publication of application:
29.11.89 Bulletin 89/48

(84) Desigheted Contrecting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: PLANT GENETIC SYSTEMS, N.V.
Kolonel Bourgstraat 106
B-1040 Brussel (BE)

(72) Inventor: Merleni, Celestina
Ellebogten 38
B-9210 Heusden (BE)

Leemans, Jan
Ph. Da Denterghemlaan 2
B-9831 Daurle (BE)

Da Greel, Willy
Coupure rechts 154
B-9000 Gent (BE)

De Beuckeleer, Marc
Fraterstraat 2
B-9220 Merelbeke (BE)

(74) Representative: Peeucelle, Chantal et al
S.C. Ernest Gutmann - Yves Plasseraud 67, bouleverd
Hausemann
F-75008 Perle (FR)

The microorganism(s) has (have) been deposited with Deutsche Sammlung von Mikroorganismen und Zellkulturen under number(s) DSM 4467, 4468, 4469, 4470, 4286, 4566, 2799.

(54) **Plants with modified stamen cells.**

(57) A plant, the nuclear genome of which is transformed with a foreign DNA sequence encoding a product which selectively disrupts the metabolism, functioning end/or development of stamen cells of the plant. The foreign DNA sequence also optionally encodes e marker.

EP 0 344 029 A1

Description

PLANTS WITH MODIFIED STAMEN CELLS

This invention relates to a male-sterile plant and to its reproduction material (e.g., seeds), in which the cells are transformed so that a foreign DNA sequence is stably integrated into their nuclear genome. The foreign DNA sequence of this invention contains et least one first foreign DNA (hereinafter the "male-sterility DNA") that: 1) encodes a first RNA or protein or polypeptide which, when produced or overproduced in a stamen cell of the plant, disturbs significantly the metabolism, functioning and/or development of the stamen cell; and 2) is in the same transcriptional unit as, and under the control of, e first promoter which is capable of directing expression of the male-sterility DNA selectively in stamen cells of the plant. In particular, this invention relates to such a nuclear male-sterile plant and its reproduction material, in which the forein DNA sequence of this invention is e foreign chimaeric DNA sequence that can also contain at least one second foreign DNA (the "marker DNA") that: 1) encodes a second RNA or protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the entire plant easily separable from other plants that do not contain the second RNA, protein or polypeptide at least in the specific tissue or specific cells; 2) is in the same transcriptional unit as, and under the control of, a second promoter which is capable of directing expression of the marker DNA in at least the specific tissue or the specific cells of the plant; and 3) is in the same genetic locus of the nuclear genome of the cells of the plant es the male-sterility DNA.

This invention also reletes to a foreign chimeeric DNA sequence that conteins at ieest one male-sterility DNA under the control of the first promoter and that can also contain, adjacent to the male-sterility DNA, at least one marker DNA under the control of the second promoter.

This invention further reletes to e vector that contains the foreign DNA sequence of this invention end is suitable for the transformation of plant cells, whereby the foreign DNA sequence is stably integreted into the nuclear genome of the cells.

This invention still further reletes to cells of a plant and to plant cell cultures, the nuclear genomes of which are trensformed with the foreing DNA sequence.

This invention yet further relates to a process for producing e nuclear male-sterile plant and its reproduction meterial and its cell cultures containing the foreign DNA sequence in which the male-sterility DNA: 1) is under the control of the first promoter and optionelly in the same genetic locus as the marker DNA under the control of the second promoter; 2) is stably integrated into the nuclear genome of the plant's cells; and 3) cen be expressed selectively in stamen cells of the plent in the form of the first RNA, protein or polypeptide.

The invention further relates to e process for producing hybrid seeds, which grow into hybrid plants, by crossing: 1) the male-sterile plant of this invention which includes, in its nuclear genome, the marker DNA, preferebly encoding a protein conferring a resistance to e herbicide on the plant; end 2) a male-fertile plant without the marker DNA in its genome. This invention particulerly reletes to such a process for producing hybrid seeds on a commercial scale, preferably in a substantially random population, without the need for extensive hand-labor.

This invention still further relates to a tapetum-specific promoter from e plent genome. This promoter can be used as the first promoter in the foreign DNA sequence of this invention for transforming the plant to render it nuclear male-sterile.

Background of the Invention

Hybridization of plants is recognized es an important process for producing offspring heving a combination of the desirable traits of the parent plants. The resulting hybrid offspring often have the ability to outperform the parents in different traits, such es in yield, edaptability to environmental changes, end disease resistance. This ability is called "heterosis" or "hybrid vigor". As a result, hybridization hes been used extensively for improving mejor crops, such es corn, sugarbeet and sunflower. For e number of reasons, primarily releted to the fact that most plents are capable of undergoing both self-pollination and cross-pollination, the controlled cross-pollination of plants without significant self-pollination, to produce e harvest of hybrid seeds, hes been difficult to echieve on a commercial scale.

In nature, the vast mejority of crop plants produce male and female reproductive organs on the same plant, usually in close proximity to one another in the same flower. This favors self-pollination. Some plants, however, are exceptions as e result of the particular morphology of their reproductive orgens which favors cross-pollination. These plants produce hybrid offspring with improve vigor and edaptebility. One such morphology in Cannabis ssp. (hemp) involves male and female reproduction organs on separate plants. Another such morphology in Zea meys (corn) involves mele and female reproductive organs on different parts of the same plant. Another such morphology in Eleels guineensis (oilpelm) involves male and fertile female gametes which become fertile at different times in the plant's development.

Some other plant species, such es Ananes comosus (pineepple), fevor cross-pollinetion through the particular physiology of their reproductive organs. . Such plants have developed e so-called "self-incompatibility system" whereby the pollen of one plant is not able to fertilize the female gamete of the same plant or of another plant with the same genotype.

Some other plant species favor cross-pollinetion by neturally displeying the so-called genomic characteristic of "male sterility". By this characteristic, the plants' anthers degenerate before pollen, produced

by the anthere, reech meturity. See: "Mele-Sterility in Higher Plants". M.L.H. Kaul, 1987, In: Monographs on Theoretical and Applied Genetics 10, Edit. Springer Verlag. Such e netural male-sterility characteristic is believed to result from a wide range of natural mutations, most often involving recessive deficiencies, and this characteristic can not easily be maintained in plant species thet predominantly self-pollinate, since under natural conditions, no seeds will be produced.

There are four main types of male sterility observed in nature. All four types of male sterility are used in commercial breeding programs to ensure that there is cross-pollination to produce hybrid seed for crops such as corn, sugarbeet, oilseed rape end sunflower.

One type of male sterility is nuclear encoded and is believed to be inherited as a recessive allele. For breeding purposee, a recessive male-sterile parent plant is maintained by crossing it with a heterozygous mele-fertile plant thet also includes the recessive male-sterility allele, so that the offspring are 50% recessive male-sterile plants. The other 50% are male-fertile plants that heve to be rogued out in outcrossing programs which can only be done efficiently if the recessive male-sterility allele is segregated together with a selectable or screenable marker. In US patent 4,727,219, e procedure is described for the use of recessive male sterility for the production of hybrid maize.

The second type of mele sterility is nuclear encoded but inherited as a dominant allele. An advantege of dominant male sterile plants, as compared to recessive male sterile plants, is thet the dominant male-sterile plants can be meintained through crossing with a male-fertile plant, to produce offspring that ere 50% dominant mele-sterile plants. The usefulness of this dominant nuclear male-sterile plant is, however, limited because its dominent male-sterility allele is in most cases not tightly linked (i.e., within the same genetic locus) to a selectable or screenable marker.

A third type of male sterility is cytoplasmatically encoded. In most cases, the cytoplasmic code is in the mitochondrial genome of the plant, end only in e few cases is the code in the chloroplast genome of the plant. The inheritance of cytoplesmatically encoded male sterility does not follow Mendelian rules but rather depends on cytoplasmic factors. The offspring obtained from crosses between cytoplasmic male-sterile plants and male-fertile plents all carry the cytoplasmic male-sterility gene and ere therefore sterile. As a result, the offspring of plents of this type are only of commercial value if the economic product of the offspring is not for use es seed but rather for plants such as ornementals and sugarbeet.

A fourth type of male sterility is the result of e combination of both nuclear encoded mele sterility and cytoplasmatically encoded mele sterility. The male sterility-inducing nuclear alleles are usually recessive, end only plants that contain the male-sterility cytoplasmic allele and that ere homozygous for the male sterility-inducing nuclear allele are phenotypically male sterile. In this type of plant, corresponding dominant male fertility-inducing alleles or "restorers of fertility", produce e male-fertile phenotype. As e result, the mele-sterile offspring of this type of plant can be mede male-fertile by pollinating the male-sterile plants with pollen conteining the restorers of fertility. As a result, the offspring of plants of this type are of commercial value where the economic product is seed, that is for plants such as corn, sorghum and sunflower.

Typically, hybrid seed production has been accomplished by the large scale planting of cytoplasmic male-sterile plants and male-fertile plants and by somehow (e.g., with a distinctive marker) preventing the resulting hybrid seeds from becoming mixed with non-hybrid seeds. According to U.S. patent no. 3,842,538, hybrid seeds are tediously seperated from non-hybrid seeds on the basis of color. According to U.S. patent no. 4,351,130, the problem of separating hybrid seeds from non-hybrid seeds is evoided by using short mele-sterile plants and tall male-fertile plants and then destroying the tall male-female plants efter pollination. According to U.S. patents 4,658,085, 4,517,763 and 4,658,084, cytoplasmic male-sterile plants are provided with a herbicide tolerance absent from the male-fertile plants which are destroyed with the herbicide after pollination. According to U.S. patent no. 4,305,225, male-sterile rice plants are spreyed with e growth hormone (e.g., gibberellin) in order to cause fuller emergence of flower-bearing panicles from rice leaf sheaths, thereby increasing the ability of the flowers to receive pollen from male-fertile plants.

In ell such processes for producing hybrid seeds from male-sterile plants, ways have been sought for simply end inexpensively obtaining on e commercial scale: 1) high hybrid seed production from eech male-sterile plant; 2) a hybrid seed population that results almost exclusively from pollen of male-fertile plants and eggs of mele-sterile plants end is substantially free of non-hybrid seeds from male-fertile plants; 3) easy production of both the mele-sterile land male-fertile plants; and 4) the virtually complete removal or destruction of either the male-fertile plants after they have pollinated the male-sterile plants or the selective separetion of non-hybrid seeds, produced by the male-fertile plants, from the hybrid seeds produced by the male-sterile plants.

Summary of the Invention

In accordance with this invention, a cell of a plant is provided, in which the nuclear genome is transformed with a foreign DNA sequence, preferably e foreign chimeeric DNA sequence, charecterized by:

(a) e male-sterility DNA encoding e first RNA, protein or polypeptide which, when produced or overproduced in a stamen cell of the plant, disturbs significantly the metabolism, functioning and/or development of the stamen cell; and

(b) a first promoter capable of directing expression of the male-sterility DNA selectively in stamen cells of the plant; the male-sterility DNA being in the same transcriptional unit as, and under the control of, the first promoter. The foreign DNA sequence in the nuclear genome of the transformed cell can also comprise, preferably in the same genetic locus as the male-sterility DNA:

(c) a merker DNA encoding a second RNA, protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the plant easily separable from other plants which do not contain the second RNA, protein or polypeptide at least in the specific tissue or specific cells; and

(d) a second promoter capable of directing expression of the marker DNA at least in the specific tissue or specific cells; the marker DNA being in the same transcriptional unit as, and under the control of, the second promoter.

Also in accordance with this invention is provided a foreign chimaeric DNA sequence that comprises the male-sterility DNA and the first promoter and that can also comprise the marker DNA and the second promoter, as well as at least one additional DNA encoding a transit peptide capable of transporting the first protein or polypeptide or the second protein or polypeptide into a chloroplast or mitochondria of a plant cell in which the foreign chimaeric DNA sequence ie expressed in its cytoplesm.

Further in accordance with this invention are provided: a male-sterile plant and a plant cell culture, each consisting of the transformed cells; a seed of the male-sterile plant; hybrid seeds and plants produced by crossing the male-sterile plant with e male-fertile plant; and e process for producing such hybrid seeds.

Still further in accordance withthis invention are provided tapetum-specific first promoters.

Description of the Invention

In accordance with this invention, a male-sterile plant is produced from e single cell of a plant by transforming the plant cell in a well known manner to stably insert, into the nuclear genome of the cell, the foreign DNA sequence of this invention. The foreign DNA sequence comprises at least one male-sterility DNA that is under the control of, and fused et its 5′ end to, the first promoter and is fused et its 3′ end to suitable transcription regulation signals (including a polyedenylation signal). Thereby, the first RNA, protein or polypeptide is produced or overproduced selectively in stamen cells of the plant so as to render the plant male-sterile. Preferably, the foreing DNA sequence also comprises et least one marker DNA that is under the control of, and is fused at its 5′ end to, the second promoter and is fused at its 3′ end to suitable transcription regulation signals (including a polyadenylation signal). The marker DNA is preferably in the same genetic locus as the male-sterility, whereby the second RNA, protein or polypeptide is produced in et least the specific tissue or specific cells of the plant so that the plant can be easily distinguished and/or separated from other plants thet do not contain the second RNA, protein or polypeptide in the specific tissue or specific cells. This guarantees, with a high degree of certainty, the joint segregation of both the male-sterility DNA and the marker DNA into offspring of the plant.

The cell of e plant (particularly a plent cepable of being infected with Agrobacterium) is preferably transformed in accordance with this invention, using a vector that is a disarmed Ti-plasmid containing the foreign DNA sequence and carried by Agrobacterium. This transformation can be carried out using procedures described, for example, in European patent publications 0,116,718 and 0,270,822. Preferred Ti-plasmid vectors contain the foreign DNA sequence between the border sequence, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such es direct gene transfer (as described, for example, in European patent publication 0,223,247), pollen mediated transformation (es described, for example, in European patent publication 0,270,356, PCT publication WO85/01856, and European patent publication 0,275,069), In vitro protoplast transformetion (es described, for example, in US patent 4,684,611), plant RNA virus-mediated transformation (as described, for example, in European patent publication 0,067,553, and US patent 4,407,956) and liposome-mediated transformation (as described, for example, in US patent 4,536,475).

Preferably, e nuclear male-sterile plant of this invention is provided by transforming a plant cell with a disarmed Ti-plasmid vector containing the foreign DNA sequence with both e male-sterility DNA under the control of a first promoter and a marker DNA under the control of a second promoter. The marker DNA can be upstreem or downstreem of the male-sterility DNA in the Ti-plasmid vector, but preferably, the two are adjacent to one another and are located between the border sequences or at least loceted to the left of the right border sequence of the Ti-plasmid vector, so that they are properly transferred together into the nuclear genome of the plant cell. However, if desired, the cell can initially be transformed with a foreign DNA sequence containing a male-sterility DNA and a first promoter and can subsequently be transformed with a marker DNA and a second promoter, inserted into the same genetic locus in the cell's nuclear genome as the male-sterility DNA. Suitable vectors for this purpose are the same as those discussed above for transforming cells with the foreign DNA sequence. The preferred vector is a disarmed Ti-plasmid vector.

The selection of the male-sterility DNA is not critical. A suitable male-sterility DNA can be selected and isolated in a well-known manner, so that it encodes the first RNA, protein or polypeptide which significantly disturbs the proper metabolism, functioning and/or development of any etamen cell in which the male-sterility DNA is expressed, preferably leading thereby to the death of any such stamen cell. Preferred examples of male-sterility DNAs encode: RNases such as RNase T1 (which degrades RNA molecules by hydrolyzing the bond after any guanine residue) and Barnase; DNases such as an endonuclease (e.g., EcoRI); or proteases such as a papain (e.g., papain zymogen and papain active protein).

Other examples of male-sterility DNAs encode enzymes which catalyse the synthesis of phytohormones, such as: isopentenyl transferase which is an enzyme that catalyzes the first step in cytokinin biosynthesis and is encoded by gene 4 of Agrobacterium T-DNA; and the enzymes involved in the synthesis of auxin and encoded by gene 1 and gene 2 of Agrobacterium T-DNA. Yet other examples of male-sterility DNAs encode:

glucanases; lipeees such as phospholipase A_2 (Verhelj et al (1981) Rev. Biochem. Pharmacol. 91, 92-203); lipid peroxidases; or plant cell wall inhibitors. Still other examples of male-sterility DNAs encode proteins toxic to plants cells, such es a bacterial toxin (e.g., the B-fragment of diphtheria toxin or botulin).

Still another example of a male-sterility DNA is en antisense DNA which encodes e strand of DNA complementary to e strand of DNA that is neturally transcribed in the plant's stamen cells under the control of an endogenous promoter es described, for example, in European patent publication 0,223,399. Such an antisense DNA can be transcribed into an RNA sequence capable of binding to the coding and/or non-coding portion of an RNA, naturally produced in the stamen cell, so as to inhibit the translation of the naturally produced RNA. An example of such an antisense DNA is the antisense DNA of the TA29 gene (described in Example 2) which is neturally expressed, under the control of the TA29 promoter, in tapetum cells of the anthers of plants.

A further example of e male-sterility DNA encodes a specific RNA enzyme (i.e., a so-called "ribozyme"), capeble of highly specific cleavege egainst a given target sequence, as described by Haseloff end Geriach (1988) Nature 334, 585-591. Such a ribozyme is, for example, the ribozyme targeted against the RNA encoded by the TA29 gene.

Still other examples of male-sterility DNAs encode products which can render the stamen cells susceptible to specific diseases, such as fungus infections. Such a male-sterility DNA can be used in a plant wherein all other cells, in which the male-sterility DNA is not expressed, are resistant to the specific disease.

By "foreign" with regard to the foreign DNA sequence of this invention is meant that the foreign DNA sequence contains e foreign male-sterility DNA and/or a foreign first promoter. By "foreign" with regard to a DNA, such es a male-sterility DNA and a first promoter, as well as marker DNA, a second promoter and any other DNA in the foreign DNA sequence, is meant thet such e DNA is not in the same genomic environment in a plant cell, transformed with such e DNA in eccordance with this invention, as is such a DNA when it is naturally found in the cell of the plant, bacteria, animal, fungus, virus, or the like, from which such a DNA originates. This means, for exemple, that a foreign male-sterility DNA or marker DNA can be: 1) a nuclear DNA in a plant of origin; 2) endogenous to the transformed plant cell (i.e., from a plant or origin with the same genotype as the plant being transformed); end 3) within the same transcriptional unit as its own endogenous promoter and 3′ end transcription regulation signals (from the plant of origin) in the foreign DNA sequence of this invention in the transformed plant cell; but 4) inserted in a different piece in the nucleer genome of the transformed plent cell than it was in the plant of origin so that it is not surrounded in the transformed plant cell by the genes which surrounded it naturally in the plant of origin. A foreign male-sterility or marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plant cell; but 3) in the same transcriptional unit as a different (i.e., not its own) endogenous promotor end/or 3′ end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign male-sterility or marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plent cell; but 3) in the same transcriptional unit as a heterologous promotor and/or 3′ end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign male-sterility or marker DNA can also, for example, be heterologous to the transformed plant cell end in the same trenscriptional unit es an endogenous promotor end/or 3′ transcription regulation signals (e.g., from the nuclear genome of a plant with the same genotype as the plant being transformed) in a foreign chimaeric DNA sequence of this invention in e transformed plant cell. An example of a foreign male-sterility DNA could come from the nuclear genome of a plant with the same genotype es the plent being trensformed and encode a catalytic enzyme, such as a protease or ribonuclease, that is endogenous to stamen cells of the plant being transformed, so thet the enzyme is overproduced in transformed stamen cells in order to disturb significantly their metabolism, functioning and/or development. Preferably, the male-sterility DNA and the marker DNA are each heterologous to the plant cell being transformed.

By "heterologous" with regard to a DNA, such es a male-sterility DNA, e first promoter, a marker DNA, a second promoter and any other DNA in the foreign DNA sequence, is meant thet such a DNA is not naturally found in the nuclear genome cells of a plant with the same genotype as the plant being transformed. Examples of heterologous DNAs include chloroplast and mitochondrial DNAs obteined from a plant with the same genotype as the plant being transformed, but preferred examples are chloroplast, mitochondrial, and nuclear DNAs from plants heving a different genotype than the plant being transformed, DNAs from enimel end bacterial genomes, and chromosomal and plasmidial DNAs from fungal and viral genomes.

By "chimeeric" with regard to the foreign DNA sequence of this invention is meant that at least one of its male-sterility DNAs: 1) is not naturally found under the control of its first promoter for the one male-sterility DNA; and/or 2) is not naturally found in the same genetic locus as at least one of its marker DNAs. Examples of foreign chimaeric DNA sequences of this invention comprise: a male-sterility DNA of bacterial origin under the control of e first promoter of plant origin; end a male-sterility DNA of plant origin under the control of e first promoter of plant origin and in the same genetic locus es e marker DNA of bacterial origin.

So that the mele-sterility DNA is expressed selectively in stamen celle of a plant, it is preferred thet the first promoter, which controls the male-sterility DNA in the foreign DNA sequence, be e promoter capeble of directing gene expression selectively in stamen cells of the plent. (By "stemen" is meant the organ of the flower that produces the male gamete and that includes an anther and a filament). Such a stamen-specific promoter can be an endogenous promoter or an exogenous promoter and can be from the nuclear genome or from the mitochondrial or chloroplast genome of e plant cell. In any event, the first promoter is foreign to the

nuclear genome of the plant cell, being transformed. Preferably, the first promoter causes the male-sterility DNA to ba exprassed only in anther, pollen or filament cells, especially in tapetum or anther epidermal cells. The first promoter can be selected and isolated in a well known manner from the species of plant, to be rendered male-sterile, so thet the first promoter directs expression of the male-sterility DNA selectively in stamen cells so as to kill or disable the stamen and render the plant incapable of producing fertile male gametes. The first promoter is preferably also selected and isolated so that it is effective to prevent expression of the male-sterility DNA in other parts of the plant that are not involved in the production of fertile polien, espacially in female organs of the plant. For example, a suitable andogenous stamen-specific first promoter can be identified and isolated in a plant, to be made male-sterile, by:

1. searching for an mRNA which is only present in the plant during the development of its stamen, preferably its anthers, pollen or filament;
2. Isoleting this stamen-specific mRNA;
3. preparing a cDNA from this stamen-specific mRNA;
4. using this cDNA as e probe to identify the regions in the plant genoma which contain DNA coding for the stamen-specific mRNA; and then
5. identifying the portion of the plant genome thet is upstream (i.e., 5') from the DNA coding for the stamen-specific mRNA and that contains the promoter of thie DNA.

Examples of such first promoters are the TA29 promoter, the TA26 promoter end the TA13 promoter, hereinafter described in the Examples, which heve been isolated from tobecco and are tapetum-specific promoters. Another tapetum-spacific first promoter from another plant species can be isolatad from its genome, using the TA29, TA26 or TA13 gene as a probe es in step 4, above. Under hybridizing conditions, such a probe will hybridize to DNA coding for e tapetum-specific mRNA in e mixture of DNA sequences from the genoma of the other plant specias (Manietis et al (1982) Molecular Cloning. A Laboratory Manuel. Ed. Cold Spring Harbor Laboratory). Theraafter, as in step 5 above, the other tapetum-specific first promoter can be identified.

If more than one male-sterility DNA is present in the foreign DNA sequenca of this invention, all the male-sterility DNAs can be under the control of e single first promoter, but preferably, each mele-sterility DNA is under the control of its own separete first promoter. Where e plurality of male-sterility DNAs are present in the foreign DNA sequence, the male-starility DNA also can encode the seme or different firet RNA(s), polypeptide(s) and protein(s). For example, when the male-sterilty DNA encodes an RNase such as RNase T1, it preferred thet at least 3, particularly 4 to 6, copies of the male-sterility DNA and its first promoter be provided in the foreign DNA sequence. In eny avent, all the male-sterility DNA(s) and their first promoter(s) ara prefarably adjacent to one enother in the foreign DNA sequance land in any vector used to transform plent cells with the foreign DNA sequence.

The selection of the marker DNA also is not critical. A suitable marker DNA can ba selacted and isolatad in a well known manner, so that it encodes e second RNA, protein or polypeptide that allows plants, expressing the marker DNA, to be easily distinguished and separated from plants not expressing the second RNA, protein or polypeptide . Examples of marker DNAs encode proteins that can provide a distinguishable color to plant cells, such as the A1 gene encoding dihydroquercetin-4-reductese (Meyer et al (1987) Nature 330, 677-678) and the glucoronidase gene (Jefferson et al (1988) Proc. Natl. Acad. Sci. USA ("PNAS") 83, 8447), or that provide e specific morphological characteristic to the plant such as dwarf growth or a different shape of tha leaves. Other examples of marker DNAs confar on plants: stress tolerance, such es is provided by the gene encoding superoxide dismutase as described in European patent application 88/402222.9; disease or pest resistance such as is provided by e gene encoding a Becillus thuringiensis endotoxin conferring insect resistence as described in European petent applicetion 86/300291.1 or a gene encoding a bacterial peptide that confers e bacterial resistance as dascribed in European patent epplication 88/401673.4

Preferred marker DNAs encode second proteins or polypeptides inhibiting or neutralizing the ection of herbicides such as: the sfr gene and the sfrv gene encoding enzymes conferring resistanca to glutamine synthetase inhibitors such as Bialephos end phosphinotricine as described in Europeen patent application 87/400,544.0; genes encoding modified target enzymes for certain herbicides that have e lower affinity for the herbicides than naturally produced endogenous enzymes, such as a modified glutamine synthetase es target for phosphinotricine as described in European petent publication 0,240,792 and e modified 5-enolpyruvylshikimate-3 phosphate synthase as e target for glyphosate as described in European petent publication 0,218,571.

The second promoter, which controls the marker DNA, can also be selected and isolated in a well known manner so that the marker DNA is expressed either selectively in one or more specific tissues or specific cells or constitutively in the entire plant, as desired depending on the neture of the second RNA, protein or polypeptide encoded by the marker DNA. For example, if the merker DNA encodes en herbicide resistance, it may be useful to heve the marker DNA expressed in all cells of the plant, using e etrong constitutive second promoter such es a 35S promoter (Odell et al (1985) Neture 313, 810-812), a 35S'3 promoter (Hull and Howell (1987) Virology 86, 482-493), the promoter of the nopaline synthetase gene ("PNOS") of the Ti-plasmid (Herrera Estralla (1983) Nature 303, 209-213) or the promoter of the octopine synthase gene ("POCS" [Da Greve et al (1982) J. Mol. Appl. Genet. 1 (6), 499-511]). If the marker DNA encodes a protein conferring disease resistance, it may be useful to have the marker DNA selectively expressed in wound tissue by using, for example, a TR promoter such as the TR1' or TR2' promoter of the Ti-plasmid (Velten et al (1984) EMBO J. 3, 2723-2730). If the marker DNA encodes e herbicide resistance, it may be useful to have the marker DNA

selectively expressed in green tissue by using, for example, the promoter of the gene encoding the small subunit of Rubisco ((European patent application 87/400,544.0). If the marker DNA encodes a pigment, it may be useful to have the marker DNA expressed in specific cells, such as petal cells, leaf cells or seed cells, preferably in the outside layer of the seed coat.

One can identify and isolate in a well known manner a tissue-specific second promoter for a plant to be rendered male-sterile and easily distinguishable from non-transformed plants by:

1. searching for an mRNA which is only present in the plant during the development of a certain tissue, such as its petals, leaves or seeds;
2. isolating this tissue-specific mRNA;
3. preparing a cDNA from this tissue-specific mRNA;
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for the tissue-specific mRNA; and then
5. identifying the portion of the plant genome that is upstream from the DNA coding for the tissue-specific mRNA and that contains the promoter for said DNA.

If more than one marker DNA is present in the foreign DNA sequence of this invention, all the marker DNAs can be under the control of a single second promoter, but preferably, each marker DNA is under the control of its own separate second promoter. More preferably, each marker DNA is under the control of its own second promoter and encodes a different second RNA, protein or polypeptide, providing different distinguishable charecteristics to a transformed plant. In any event, the merker DNA(s) and second promoter(s) should be adjacent to each other and to the one or more male-sterility DNAs contained in the foreign DNA sequence of this invention and in any vector used to transform plant cells with the foreign DNA sequence.

It is generally preferred that the first RNA, protein or polypeptide, encoded by the male-sterility DNA, interfere significantly with the stamen cells' metabolism, functioning and/or development by acting in the cytoplasm or the nucleus of the stamen cells. However, when it is desired to have the first protein or polypeptide and/or of the second protein or polypeptide transported from the cytoplasm into chloroplasts or mitochondrie of the cells of transformed plants, the foreign DNA sequence can further include an additional foreign DNA encoding e transit peptide. The additional DNA is between the male-sterility DNA and the first promoter is the first protein or polypeptide is to be so-transported and is between the marker DNA and the second promoter if the second protein or polypeptide is to be so-transported. By "transit peptide" is meant a polypeptide fragment which is normally associated with e chloroplast or mitochondrial protein or subunit of the protein and is produced in a cell as a precursor protein encoded by the nuclear DNA of the cell. The transit peptide is responsible for the translocation process of the nuclear-encoded chloroplast or mitochondrial protein or subunit into the chloroplast or the mitochondrial, and during such e process, the transit peptide is separated or proteolytically removed form the chloroplast or mitochondrial protein or subunit. One or more of such additional DNA's can be provided in the foreign DNA sequence of this invention for transporting one or more first or second proteins or polypeptides as generally described in European patent applications 85/402,596.2 and 88/402,222.9 and in: Van den Broeck et al (1985) Nature 313, 358-363; Schatz (1987) Eur. J. of Bioch. 165, 1-6; and Boutry et al (1987) Nature 328, 340-342. An example of a suitable transit peptide for transport into chloroplasts is the transit peptide of the small subunit of the enzyme RUBP carboxylase (European patent application 85/402,596.2) and an example of a transit peptide for transport into mitochondria is the transit peptide of the enzyme Mn-superoxide dismutese (see Example 16).

In the foreign DNA sequence of this invention, 3' transcription regulation signals can be selected among those which are capable of enabling correct transcription termination and polyadenyletion of mRNA in plant cells. The transcription regulation signals can be the natural ones of the gene to be transcribed but can also be foreign or heterologous. Examples of heterologous transcription regulation signals are those of the octopine synthase gene (Gielen et al (1984) EMBO J. 3,, 835-845) and the T-DNA gene 7 (Velten end Scheil (1985) Nucleic Acids Research ("NAR") 13, 6981-6998).

Also in accordance with this invention, plant cell cultures, such as anther cell cultures, containing the foreign DNA sequence of this invention in which the first promoter effects expression of the male-sterility DNA at a given stage of pollen development, more especially after meiosis, can be used to regenerate homozygous dominant male-sterile plants ("Efficient isolation of microspores and the production of microspore-derived embryos from Brassica napus", E.B. Swanson, M.P. Coumans, S.C. Wu, T.L. Barby and W.D. Beversdorf, Plant Cell Reports (1987) 6: 94-97).

Further in accordance with this invention, processes are provided for producing hybrid seeds which can be grown into hybrid plants. One process involves crossing a nuclear male-sterile plant including at least one marker DNA with a male-fertile plant without the marker DNA. Both male-sterile and male-fertile plants are planted in separate rows near to each other. Another process involves crossing a nuclear male-sterile plant including at least two different marker DNAs with a male-fertile plant including, in common, only one of the two different marker DNAs in a homozygous form. Both male-sterile and male-fertile parent plants can be grown in a substantially random population, increasing the chances of cross-pollination, without the need for precise planting patterns. The male-fertile parent plant can thereafter be easily removed from the population, using the distinctive trait encoded by the non-common marker DNA which is not possessed by the male-fertile parent plant. Preferably in this process, the non-common marker DNA in the male-sterile plant is under the control of a constitutive promoter and encodes a protein or polypeptide that renders the male-sterile plant resistant to a particular herbicide. The male-fertile plant can then be destroyed after cross-pollination, using the particular

herbicide.

Plants, transformed with the male-sterility DNA, preferably with both the male-sterility DNA and the marker DNA encoding herbicide-resistance, stably integrated and transmissible throughout generations as dominant alleles in accordance with this invention, are alternatives to, and provide several advantages over, presently used cytoplasmic male-stenlity systems for breeding and producing hybrid crops. Such advantagee include:

1. For cross-pollinating crops, the breeding strategy is much simplified, because it is not necessary to introduce a restorer gene into the male-fertile parent line of the cross that will produce the commercially sold hybrid seed. Indeed, a heterozygous nuclear male-sterile parent line crossed with another male-sterile parent line for commercial seed production will produce 50% male-sterile hybrid offspring and 50% male-fertile hybrid offspring, as a result of which the commercial crop will produce enough pollen to guarantee full seed set and therefore normal yield. Examples for such crops are corn and oilseed rape.

2. For crops for which the seeds do not represent the economic harvest, the breeding strategy is also much simplified without the need of a restorer gene expressed in the male-fertile perent line. Indeed, for these crops it does not matter that 50% of the commercially sold hybrid seeds are male-sterile. Examples for these crops are sugarbeet and alfalfa.

3. The system allows production of nuclear male-sterile lines end maintainer lines from existing inbred lines in one operation, eliminating the need for backcrossing. This reduces the time lag between conception and commercialization of a hybrid by at least 6 to 8 generations. An example of e typical strategy for producing hybrid plants using es parent plant the plants heving inserted and expressing the male-sterility DNA may consist of the following steps:

1) making test hybrids by hand, by crossing inbred lines, and testing for combining ebility end selected characteristics (2 years).

2) making one parent line of each of the selected hybrids nuclear male-sterile by the process which is the object of this invention (1 year).

3) multiplying the nuclear male sterile parent plant obtained from said process, hereinafter called "A^S", and its maintainer line, hereinafter called "A", and the pollinating male-fertile parent plant, hereinafter called "B", of the future commercial crop (3 years). During the seme period, introducing the selected hybrids in official yield trials (3 years).

4) producing and selling the approved hybrid seed (1 year).

4. Combined with a marker DNA encoding herbicide-resistance, such a nuclear male-sterility system allows production of 2-, 3- and 4- way hybrids in any combination required. It is believed to be sufficient to introduce the male-sterility DNA and adjacent thereto the marker DNA into the nucleer genome of one plant which will be used as one of the grandparent breeding lines for obtaining 2- or 3-way hybrids, and into the nuclear genome of two plants which will be used as the two grandparent lines for 4-wey hybrids. Each breeding line can be maintained by the following two crosses given here by way of example, and whereby "SH" stands for the dominant alleles respectively of male-sterility (S) and herbicide resistance (H), and sh stands forthe recessive alleles respectively of male fertility (s) and herbicide sensitivity (h):

a. SH/sh x sh/sh gives 50% SH and 50% sh offspring, and after spraying with the herbicide to which H confers resistance, 100% sterile seedlings are obtained.

b. sh/sh x sh/sh gives 100% fertile offspring.

5. It provides a protection for the owner of the marker DNA that has been integrated into the male-sterility system by making it more difficult for competitors to breed the marker DNA into their own breeding lines.

For illustrative purposes, two crop breeding schemes in accordance with this invention are given as follows:

Scheme 1: Breeding a plant containing adjacent male-sterility DNA and marker DNA encoding herbicide-resistence

1A) maintaining the male-sterility line A^S:
line $A^{SH/sh}$ x line $A^{sh/sh}$
giving
50% $A^{SH/sh}$ (phenotype: male-sterile, herbicide-resistant)
50% $A^{sh/sh}$ (phenotype: male-fertile, herbicide-susceptible)

1B) producing the hybrid seed crop:

a) planting seeds of $B^{sh/sh}$ (male plants) and the seeds obtained by the cross 1A) consisting of $A^{SH/sh}$ and $A^{sh/sh}$ ("female" plants) in separate rows.

b) eliminating the genotype $A^{sh/sh}$ by spraying the female rows with the herbicide.

c) cross-pollination occurring:
$A^{SH/sh} \times B^{sh/sh} \times B^{sh/sh}$
giving in the female rows:
50% $AB^{SH/sh}$ (phenotype: hybrid, male-sterile, herbicide-resistant)
50% $AB^{sh/sh}$ (phenotype: hybrid, male-fertile, herbicide-sensitive)

and in the male rows: 100% $B^{sh/sh}$.

d) eliminating the genotype $B^{sh/sh}$ occurring in the male rows by spraying with the herbicide or by mechanical means.

e) harvesting the hybrid seeds of the female rows wherein the cross-pollination of c) occurred This is the commercially sold seed.

Scheme 2: Breeding a plant containing adjacent male-sterility DNA and two marker DNAs, each encoding a different herbicide-resistance (H1 and H2).

2A) maintaining the male-sterile line A^S:
A^S:$A^{SH1H2/sh1h2}$ x $A^{sh1h2/sh1h2}$
giving
50% $A^{SH1H2/sh1h2}$ (phenotype: male-sterile, resistant to both herbicides).
50% $A^{sh1h2/sh1h2}$ (phenotype: male-fertile, susceptible to both herbicides).

2B) maintaining pollination line B:
$B^{sh1H2/sh1H2}$ X $B^{sh1H2/sh1H2}$
giving
100% $B^{sh1H2/sh1H2}$ (phenotype: male-fertile, susceptible to herbicide 1 and resistant to herbicide 2).

2C) producing the hybrid seed crop:

a) planting the seeds obtained from 2A) and the seeds obtained from 2B) at random.

b) eliminating the genotype $A^{sh1h2/sh1h2}$ by spraying the field with herbicide 2.

c) cross-pollination occurring:
$A^{SH1H2/sh1h2}$ x $B^{sh1H2/sh1H2}$
giving
50% $AB^{SH1H2/sh1H2}$
50% $AB^{sh1h2/sh1H2}$
and
self-pollination occurring:
$B^{sh1H2/sh1H2}$ x $B^{sh1H2/sh1H2}$
giving
100% $B^{sh1H2/sh1H2}$

d) eliminating plants with genotype $B^{sh1H2/sh1H2}$ obtained from the parent line B, for which self-pollination occurred, by spraying the field with herbicide 1.

e) harvesting hybrid seeds of the remaining plants $A^{SH1H2/sh1H2}$ obtained by the cross-pollination of c).

The following Examples illustrate the invention. The figures referred to in the Examples are as follows:

Fig. 1. shows restriction maps of TA29 cDNA and its ClaI fragment in pTA29S3 of Example 1.

Fig. 2 shows the cDNA sequence of the PstI fragment of the TA29 gene of Example 2.

Fig. 3A shows the DNA sequence and amino acid sequence of the TA29 gene, from its ClaI site to its HindIII site. Above the sequences, the important restriction sites are indicated, and under the sequences is the amino acid sequence encoded by the ORF. Also indicated are:
- from nucleotide ("nt") 1446 to 1452: TATA box (asterisks),
- at nt 1477: transcription initiation site of TA29 mRNA (asterisk),
- from nt 1514 to 1537: the 3' to 5' sequence of a synthetic oligomer as described in Example 2, and
- from nt 1940 to 2296 (between arrows); the aligned sequence of TA29 cDNA.

Fig. 3B shows the alignment of the TA13 cDNA (top line) and the TA29 cDNA (bottom line); as discussed in Example 4. Homologous nucleotides are indicated by vertical lines.

Fig. 3C shows the sequence of the TA26 cDNA, as discussed in Example 4; the ORF is underlined.

4A shows schematically the construction of the vector pMB2 of Example 3.

Fig. 4B shows a map of the vector pMB3 of Example 3.

Fig. 5 shows a map of the vector pTTM3 of Example 5.

Fig. 6 shows a map of the vector pTTM4 of Example 7.

Fig. 7A shows a map of the vector pTTM6 of Example 9.

Fig. 7B shows a map of the vector PTTM6A$^-$ of Example 11.

Fig. 8 shows a map of the vector pTTM8 of Example 12.

Fig. 9A shows a map of the vector pTVEP1 of Example 14.

Fig. 9B shows a map of the vector pTVEP2 of Example 14.

Fig. 10A shows a map of the vector pTVEP63 of Example 16.

Fig. 10B shows a map of the vector pTVEP62 of Example 16.

Fig. 11 shows a photograph of flowers of normal tobacco plants compared with flowers of tobacco plants transformed with the male-sterility DNA of Example 9.

Fig.12 shows a photograph of a transverse cutting of the anther of a normal tobacco plant compared with the anther of a tobacco plant transformed with the male-sterility DNA of Example 9 (enhancement: x 250).

Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA were carried out by the standardized procedures described in Maniatis et al, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory (1982). The following plasmids and vectors, used in the Examples, have been deposited in the Deutsche Sammlung Für Mikroorganismen und Zellcuituren ("DSM"), Mascheroder Weg 1B, D-330 Braunschweig, Federal Republic of Germany under the provisions of the Budapest Treaty:

Plasmid or vector	DSM Accession No.	Date
pMB3	4470	2t Mar. 1988
pGSC1600	4467	21 Mar. 1988
pGCC1700	4469	21 Mar. 1988
pGV2260	2799	Dec. 1983
pGSC1701A	4286	22 Oct. 1987
pTTM4	4471	2t Mer. 1988
pMAC5-8	4566	25 April 1988
pTTM6	4468	2t Mar. 1988

Example 1 - Subcloning of an anther-specific gene (the "TA29 gene")

From Professor Robert Goldberg of the University of California, Los Angeles (UCLA) were obtained: a Nicotiana tabacum anther-specific cDNA ("TA29 cDNA") cloned as a PstI fragment in pBR329 (Covarrubias end Bolivar (1982) Gene 17, 79) by GC tailing; and the corresponding genomic clone ("lambda TA29") that was isolated from a N. tabacum "Samsun" genomic library using TA29 cDNA as a probe and that was inserted in the EcoRI site of the lambda phage vector cH32 (Loenen and Blattner (1983) Gene 26, 17t). The TA29 cDNA was 365 base pairs long (±0.4 kb) and hybridized to a tapetum-specific mRNA of 1,100 nucleotides which accounts for 0.24% of the poly A+ mRNA from anthers of the N. tabacum. As shown in Fig. t, lambda TA29 contains two EcoRI fragments, the total insert measuring 13.2 kb.

An internal 7.5 kb ClaI fragment as shown in Fig. 1, containing the TA29 gene, was subcloned from lambda TA29 in pLK3t (Botterman and Zabeau (1987) DNA 6, 6) which produced a plasmid names "pTA29S3". Nitrocellulose bound fragments of lambda TA29, digested with the combination of EcoRI/ClaI/HindIII/HindIII-EcoRI and the combination of ClaI-EcoRI end hybridized egainst TA29 cDNA, indiceted the presence of sequences homologous to TA29 cDNA.

Example 2 - Nucleotide sequence determination of TA29 cDNA and its homologous sequence from pTA29S3; mapping of TA29 gene and its promoter.

The PstI insert of TA29 cDNA in pBR329 was completely sequenced (Maxam and Gilbert (1977) Proc. Natl. Acad. Scl. USA ("PNAS") 74, 560). The cDNA sequence is shown in Fig. 2. It reveals the presence of one open reading frame over the entire cDNA sequence (as indicated).

Then, the sequence of the ClaI insert in pTA29S3 was determined from the ClaI site to the HindIII site (3261 base pairs apart). Comparison of the TA29 cDNA sequence and the pTA29S3 sequence revealed the presence of a sequence in the pTA29S3 which wes completely homologous with the TA29 cDNA sequence.

Figure 3 shows the sequence of the TA29 gene in pTA29S3. The sequence in pTA29S3 that is identical to the TA29 cDNA sequence is between the arrows in Fig. 3. A putative open reading frame is revealed by the corresponding amino acid sequence in Fig. 3. This indicates thet the TA29 gene encodes e protein of 321 amino acid residues and thet there are no introns present in the coding region. The length of the open reading frame of 964 (+ leader) nucleotides matches the size of a transcript present in tobacco anther mRNA prepared from anthers isolated from young (12-20 mm long) tobacco flower buds and absent in the mRNA isolated from leaf and older flowers (when the buds are opened and petals have appeared). The size of this mRNA is approximately t t00 nucleotides.

There are two ATG codons, one et nucleotide ("nt") t527 and the other at nt 1560, which could serve es initiation codon for the open reading frame, 33 nucleotides apart. There is a consensus sequence TATA at nt 1446 present 81 nucleotides 5′ upstream of the first ATG codon (indicated by asterisks in Fig. 3). To confirm that this "TATA" box is part of the promoter of the TA29 gene, the 5′ end of the TA29 mRNA wes determined. This was done by primer extension (Mc Knight et al (198t) Cell 25, 385). For this purpose, an oligomer of 24 nucleotides, having the sequence: 5′ GGA GCT ACC ATT TTA CGT AAT TTC 3′, was used as it is complementary to the TA29 gene from nt 1514 to nt 1537 es shown in Fig. 3.

This oligonucleotide was ^{32}P labeled by kinetion at the 5′ end. After being hybridized with anther mRNA, the oligonucleotide was extended by reverse transcriptase. The resulting extended oligonucleotide was analyzed on a sequencing gel, next to a sequencing ladder, to determine its exact size. The fragment was shown to be 61 nucleotides long. This indicates that transcription initiation of the TA29 mRNA occurred at nt 1477 (indicated by asterisk in Fig. 3). Hence, the TA29 gene has a TATA box located 31 nucleotides upstream of the transcription initiation site. The mRNA contains a 51 nucleotide-long leader sequence from nt t477 to nt 1527,

a coding region of 964 nucleotides from nt 1527 to nt 2491, and a 3′ non coding region of approximately 100 nucleotides from nt 2492 to nt 2590. As is the case in approximately 92% of presently characterized plant genes (Joshin (1987) Nucleic Acids Research ("NAR") 15 (16), 6643), it is believed that the first AUG codon of the mRNA is used to initiate translation. The TA29 promoter thue appears to be located between the ClaI restriction site and nt 1477.

Example 3 - Construction of e promoter cassette ("PTA29") derived from the TA29 gene

To construct chimaeric DNA sequences containing the 5′ regulatory sequences, including the promoter, of the TA29 gene in the same transcriptional unit as, and controlling, a first heterologous male-sterility DNA, a cassette was constructed as shown in Fig. 4 by eubcloning a 2.5 kb ClaI/AccI fragment from pTA29S3 into the polylinker AccI site of pMAC 5-8 (European patent application 87/402348.4). This produced a vector named "pMB2", shown in Fig. 4, which could be used to isolate single strand DNA for use in site directed mutagenesis.

Then, the sequence surrounding the first ATG codon AAAATGGTA was modified to ACCATGGTA by substituting two adenine residues for cytosine residues. This mutation created the sequence CCATGG which is the recognition site for the restriction enzyme NcoI. This site directed mutagenesis in pMB2 was performed using e synthetic oligonucleotide of 24 nucleotides with the following sequence:

3′GTT TAA TCG ATG GTA CCA TCG AGG 5′

The resulting plasmid, containing the newly created NcoI site, was named "pMB3" and is shown in Fig. 4 bis. The precise nucleotide sequence spanning the NcoI site was determined in order to confirm that it only differed from the 5′ sequence of the TA29 gene by the AA -- CC substitution, creating the NcoI site. The 1507 nucleotide long fragment ClaI -- NcoI was named "PTA29".

Example 4 - Identification of cDNA clones obtained from other stamen-specific mRNAs

To demonstrate that other anther-specific mRNAs could be identified end then used to isolate cDNA clones with analogous properties to the TA29 gene, two other N. tabacum anther-specific cDNAs ("TA13 cDNA" and "TA26 cDNA") were obtained from Professor Goldberg of UCLA.

TA13 cDNA is a clone of 1100 bp which hybridized to two mRNA species of about 1100 and 1200 nucleotides, respectively, which are specific for tapetum cells and are abundent at a very early stage of enther development. TA13 cDNA was sequenced, using the procedure of Example 2, and then compared with the sequence of TA29 cDNA as shown in Fig. 3B. This sequence comparison reveals that TA13 cDNA end TA29 cDNA share 92% homology, and the ORF is very rich in glycine content.

TA26 cDNA was cloned as a PstI insert into pBR329 by poly-G/C tailing. It is a clone of 519 bp which hybridized to one tobacco mRNA species of 580 nucleotides, which mRNA is specific for tapetum cells end ebundent at a certain stage of anther development. The entire TA26 cDNA was sequenced, using the procedure of Example 2, and when compared with the sequence of TA29 cDNA, revealed no homology. The sequence of TA26 cDNA is given in Fig. 3C.

Example 5 - Construction of e chimaeric DNA sequence of PTA29 and a glucuronidase gene

A plasmid named "pTTM3", shown in Fig. 5, was constructed by assembling the following well known DNA fragments:

1. e vector fragment, including T-DNA border sequences, derived from pGSC1600;
2. a chimaeric sequence containing the promoter cassette PTA29 from Example 3, fused in frame with a pMB3 NcoI/EcoRI fragment containing an E. coli gene encoding beta-glucuronidase ("GUS" [Jefferson et al (1986) PNAS 83, 8447; Jefferson et al (1987) EMBO J. 6, 3901]) and the 3′ end signals of an octopine-synthase gene ("OCS" [Dhaese et al (1983) EMBO J. 2, 419]);
3. a chimaeric sequence containing an Arabidopsis SSU promoter ("PSSU") or "PSSUARA), a herbicide resistance gene sfr (European patent application 87/400,544.0) end the 3′ end signals of a T-DNA gene 7 (Velten and Schell (1985) NAR 13, 6981); and
4. A chimaeric sequence containing the EcoRI/SacI fragment from pGSFR401 which contains a nopaline-synthase promoter ("PNOS"), a neo gene encoding kanamycin resistance and the 3′ end signals of an octopine synthase gene (European patent application 87/400,544.0, wherein pGSFR401 is called "pGSR4"). pTTM3 is a T-DNA vector containing, within the T-DNA border sequences, two chimaeric sequences: PSSU-sfr in which the sfr is e merker DNA (European patent application 87/400,544.0) under the control of PSSU as a second promoter; and PTA29-GUS in which GUS is a reporter gene whose expression in plants and plant cells under the control of the TA29 promoter can easily be localized and quantified.

Example 6 - Introduction of the chimaeric DNA sequence of Example 5 into tobacco

A recombinant Agrobacterium strain wes constructed by mobilizing pTTM3 (from Exemple 5) from E. coli into Agrobacterium C58C1 Rif^R containing pGV2260 (De Blaere et al (1985) NAR 13, 4777). Mobilization was carried out using E. coli HB101 containing pRK2013 (Figurski et al (1979) PNAS 76, 1648) as a helper as described in European patent publication 0,116,718. The resulting Agrobacterium strain contained a hybrid Ti-plasmid comprising pGV2260 and pTTM3.

This strain was used to transform tobacco leaf discs (N. tabacum Petite Havane SR1) using standard procedures as described, for example, in European patent application 87/400,544.0. Transformed calli and

shoots were selectad using 5 mg/l of tha herbicide phosphinothricin in tha madium (De Block et al (1987) EMBO J. 6, 2513). No beta-glucuronidasa enzyme activity was datectad in the transformed herbicide-resistant calli and shoots.

Then, the transformed shoots were rootad, transferred to soil in the greanhouse and grown until they flowered. The flowers were examined, and only the tapetum cells in tha anthers of the stamen were found to contain beta-glucuronidasa activity. This shows that the TA29 promoter is capable of directing expression of a heterologous gene, lika tha bata-glucuronidase gena, selactively in tapatum calls of the plants.

Example 7 - Construction of a chimaeric DNA sequence of PTA29 and a gena 4

A plasmid named "pTTM4", shown in Fig. 6, was constructed by assembling tha following well known DNA fragments:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1700 (Cornelissen and Vandewiele (1989) NAR 17 (1), 19-29);
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter controlling expression of herbicide-resistanca gene sfr and the 3′ end of a T-DNA gene 7;
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter controlling expression of the neo gene and the 3′ end of the octopine synthase gene; and
4. a chimaeric sequence containing the PTA29 promoter cassette from Example 3, fused in frame with an Agrobacterium T-DNA gena 4 that encodes isopantenyl transferasa (Akiyoshi et al (1984) PNAS 76, 5994; Barry et al (1984) PNAS 81, 4776) containing its own 3′ end transcription regulation signals. pTTM4 is a binary type T-DNA vector containing, within the T-DNA border sequences, the following chimaaric sequences: PSSU-sfr and PNOS-neo in which tha sfr and neo genas ara marker DNAs that encoda dominant selectable markers for plants and that ara under control of respactively PSSU and PNOS as sacond promoters; and PTA29-gena 4 in which gene 4 is a male-sterility DNA that is under the control of PTA29 as a first promoter and encodes tha enzyme isopentenyl transferase which will cause the enhanced production of cytokinin. Enhanced cytokinin production in tapetum cells, under the control of the TA29 promoter, will disturb the metabolism and organogenesis of the tapetum cells.

Example 8 - Introduction of the chimaeric DNA sequence of Exampia 7 into tobacco

As described in Example 6, pTMM4 (from Example 7) was introduced with mobilization from E. coli into Agrobacterium C58C1 Rif^R. The resulting Agrobacterium strain contained a binary type Ti-plasmid comprising pGV2260 and pTTM4.

As also described in Exampia 6, this strain was usad to transform tobacco leaf discs, and transformad calli and shoots were selected using 5 mg/l of phosphinothricin. Transformed herbicide-resistant shoots were rooted; which shows that gene 4 was not yet being expressed in the transformed plants.

The plants were then transferrad to soil in the greanhousa and grown until thay flower. The flowers are examined, and no functional tapetum cells are found in their anthers of their stamen. This shows that the TA29 promoter is capeble of directing expression of the haterologous gene 4 selectively in tapetum cells of the plants.

Example 9 - Construction of a chimaeric DNA sequence of PTA29 and a RNAse T1 gene

A plasmid named "pTTM6", shown in Fig. 7A, was constructed by assembling the following well known DNA fragments:

1. a vector fragment, including T-DNA border sequences, from pGSC1600;
2. the chimeeric sequence (no. 3) of Exampia 5, containing the PSSU promoter, the herbicide resistance gene sfr and the 3′ end of tha T-DNA gene 7; and
3. a chimaeric saquence, containing the pTA29 promoter cassette from Example 3, fused in frame with a synthatic gene encoding RNasa T1 from A. orhyzae, (Quaas at al, "Blophosphates and their Analogues-Synthese, Structure, Metabolism and Activity" (1987) Elsevier Science Publisher B.V., Amsterdam; Quaas et al (1988) Eur. J. Biochem. 173, 617-622.) and the 3′ end signals of a nopaline synthase ("NOS") gena (An et al (1985) EMBO J. 4 (2), 277).

pTTM6 is a T-DNA vactor containing, within tha T-DNA bordar sequences, two chimaeric saquances; PSSU-sfr which is a marker DNA under the control of PSSU as e second promoter; and PTA29-RNase T1 gene which is a male-sterility DNA under the control of PTA29 as a first promoter. Exprassion in tapetum cells of the male-sterility DNA under the control of tha TA29 promoter will produce RNasa T1 that will ba lethal for the cells, since the RNase T1 will degrade the RNA molecules which are indispensable for these cells' metabolism.

Exampia 10 - Introduction of the chimaaric DNA sequance of Example 9 into tobacco

As described in Example 6, a recombinant Agrobacterium strain was constructed by mobilization of pTTM6 (from Exampia 9) from E. coli into Agrobacterium C58C1 Rif^R. The resulting Agrobacterium strain, harboring a cointagrated Ti-plasmid comprised of pGV2260 and pTTM6, was usad for transforming tobacco leaf discs. Transformed calli and shoots wera selected using 5 mg/l phosphinothricin. That the RNase T1 gene was not expressad in the transformed herbicide-rasistant calli and shoots was shown by their growth.

The transformed shoots wera rooted, transferred to soil in tha graanhouse and grown until they flowerad. The transformed tobacco plants developed normal flowers except for their anthers. The anthers, although of

normal shepe, dehisched leter in time, compared to the anthers of non-transformed tobacco plante (see Fig. 11). Upon dehiscense, either little or no pollen was released from the transformed plants, and the pollen grains formed by the transformed plants, were about 50 to 100 times smeller in volume than normel pollen grains and were irregularly sheped. Moreover, most of the pollen grains from transformed plants failed to germinete, and the germinetion efficiency of pollen from transformed plants was about 0 to 2% of the germination efficiency of normal pollen grains. Furthermore, the transformed plants did not produce any seeds by self-pollinetion -- neither by netural self-pollination nor by hand-provoked self-pollinetion.

Microscopic evaluetion, by thin layer cross section, of e transformed plant showed thet no normal tapetum leyer was formed and thet the pollen seck remeined empty (see Fig. 12). This shows thet the TA29 promoter is cepeble of directing expression of the heterologous RNase T1 gene selectively in tapetum cells of the transformed plants, and thet the RNese T1 is capeble of sufficiently disturbing the functioning of the tapetum cells, so es to render the plants male-sterile.

Example 11 - Introduction of e derivative of the chimaeric DNA sequence of Example 9 into oilseed rape

A recombinant Agrobacterium strain was constructed by mobilization of pTTM6A$^-$ from E. coli into Agrobacterium C58 Rif^R containing pMP90 (Koncz and Schell (1986) Mol. Gen. Genetics 204, 383-396). pMP90 provides vir and trans functions end does not carry e gene encoding ampicillin resistance. As shown in Fig. 7B, pTTMA6A$^-$ is e derivetive of PTTM6 (from Example 9), in which the β-lactamese gene encoding ampicillin resistance has been inactivated by insertion of e DNA sequence into the ScaI site of the β-lactamase gene.

The resulting Agrobacterium strain (named "A3144"), harboring pMP90 end pTTM6A$^-$, was used for the transformetion of Brassice napus according to the procedure of Lloyd et al (1986) Science 234, 464-466 and Kilmaszewska et al (1985) Plent Cell Tissue Organ Culture 4, 183-197. Cerbenicilin was used to kill A3144 efter co-cultivation occurred. Transformed calli were selected on 5 mg/l phosphinotricine and 100 ug/ml kanamycin, and resistant calli were regenerated into plents. After induction of shoots and roots, the transformants were transferred to the greenhouse and grown until they flower. The flowers ere examined, end they exhibit essentially the same phenotype as was observed for the transformed tobecco plants described in Example 10. This shows thet the TA29 promoter is cepable of directing the expression of the heterologous RNase T1 gene selectively in tepetum cells of plents other then tobecco, so es to render such other plants male-sterile.

Example 12 - Construction of e chimeeric DNA sequence of PTA29 and e Bernase gene

A plasmid named "pTTM8" shown in Fig. 8, was constructed by essembling the following well known fragments:

1. e vector fragment, including T-DNA border sequences derived from pGSC1700 (Cornelissen and Vandewiele (1989) NAR 17 (1) 19-29) and in which the β-lactemase gene (1' of Fig. 8) has been inactivated by insertion of e DNA sequence into its ScaI site;
2. the chimeeric sequence (no. 3) of Exemple 5, containing the PSSU promoter, the herbicide-resistance gene sfr end the 3' end of T-DNA gene 7;
3. the chimeeric sequence (no. 4) of Exemple 5, conteining the PNOS promoter, the neo gene, and the 3' end of the octopine synthase gene; and
4. e chimaeric sequence, containing the PTA29 promoter cassette from Exemple 3, fused in frame with the Bernese gene from Bacillus emiloiquefaciens (Hartley and Rogerson (1972) Preparative Biochemistry 2, (3), 243-250) and the 3' end of the nopaline synthase gene of Example 9.

pTTM8 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimeeric sequences: PSSU-sfr and PNOS-neo which are markers DNAs with respectively PSSU and PNOS es second promoters; and PTA29-Barnase gene which is e male-sterility DNA under the control of PTA29 es e first promoter. Expression in tepetum cells of the male-sterility DNA under the control of the TA29 promoter will produce Barnese selectively in the tapetum cells so that Barnase will interfere with the metebolism of these cells.

Example 13 - Introduction of the chimaeric DNA sequence of Example 12 into tobacco and oilseed repe

As described in Example 11, e recombinant Agrobacterium strain was constructed by mobilizing pTTM8 (from Example 12) from E. coli into Agrobecterium C58C1 Rif^R conteining pMP90 (Koncz and Schell (1986) Mol. Gen. Genetics 204, 383-396). The resulting strain (named "A3135"), harboring pMP90 and pTTM8, is used for tobecco leef disc transformetion and for oilseed rape transformation. Transformed calli and shots are selected using 5mg/l phosphinothricin end 100 ug/ml kanamycin. That the Bernase gene is not expressed in the transformed herbicide-resistant calli and shoots is shown by their growth.

The transformed shoots ere rooted, transferred to soil in the greenhouse end grown until they flower. The flowers of both the tobecco and oilseed repe are examined, and e phenotype is observed for the transformed plants thet is essentially the same es the phenotype of the traneformed tobecco plents described in Exemple 10. This shows thet the TA29 promoter is capable of directing expression of the heterologous Bernase gene selectively in tapetum cells of the plants, thereby rendering the plants male-sterile.

Example 14 - Construction of e chimeeric DNA sequence of pTA29 and a gene encoding pepain

A plasmid named "pTVEP1", shown in Fig. 9A, is constructed by essembling the following well known fragments:

1. a vector fragment, including T-DNA border sequences derived from pGSC1700 and in which the β-lactamase gene (1′ of Fig. 9A) has been inactivated by insertion of e DNA sequence into its ScaI site;
2. the chimeeric sequence (no. 3) of Example 5, containing the PSSU promoter, the herbicide resistance gene sfr and the 3′ end of T-DNA gene 7.
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene and the 3′ end of the octopine synthase gene; and
4. a chimeeric sequence, containing the PTA29 promoter cassette from Example 3, fused in frame with:
a) a papain gene from Carica papeya fruit, encoding the papain zymogen which is a plant endopeptidase (Cohen et al (1986) Gene 48, 219-227) capable of attecking peptide, as well as ester, bonds; the following modifications are made in the DNA sequence of Cohen et al (1986) using site directed mutagenesis as described in Example 3:
I. the nucleotide A, position-1 upstream of the first ATG codon, is mutated into nucleotide C in order to obtain a suitebie NcoI cloning site; and
ii. the GAA codons encoding glutamate at positions 47, 118, 135, respectively, are muteted into CAA codons encoding glutamine; and
b) the 3′ end of the nopeline synthase gene of Example 9.

pTVEP1 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimeeric sequences: PSSU-sfr and PNOS-neo which are marker DNAs encoding dominant selectable markers for plant transformations, under the control of respectively PSSU and PNOS as second promoters; and PTA29-Papain gene which is e male-sterility DNA under the control of PTA29 es e first promoter. Expression in tepetum cells of the male-sterility DNA under the control of the TA29 promoter will produce an endopeptidase (the papain zymogen) thet will cleave proteins in the tapetum cells, thus leading to the death of these cells.

A plasmid name "pTVEP2", shown in Fig. 9B, is elso constructed by assembling the following well known fragments:

1. a vector fragment, including T-DNA border sequences derived from pGSC1700 and in which the β-lactamase gene (1′ of Fig. 9B) has been inactivated by insertion of a DNA sequence into the ScaI site;
2. the chimaeric sequence (no. 3) of Example 5, conteining the PSSU promoter, the herbicide resistance gene sfr and the 3′ end of T-DNA gene 7;
3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene, and the 3′ end of the octopine synthase gene; and
4. e chimaeric sequence, containing the PTA29 promoter cassette of Exemple 3, fused in frame with: e) a papein gene from Carice papaya fruit, encoding the active protein of the papain zymogen; the following modifications are made in the DNA sequence of Cohen et al (1986), using site directed mutegenesis as described in Example 3:
i. the AAT codon encoding Asn, upstream of the first Ile residue of the active protein, is mutated into a GAT codon, which provides a suitable EcoRV cloning site (GAT ATC). The EcoRV engineered site is fused directly to the pTA29 cassette in order to obtain a direct in frame fusion of the promoter with the sequence encoding the active protein of the papain zymogen; end
ii. the GAA codons encoding glutamate et positions 47, 118, 135 respectively, are mutated into CAA codons encoding glutamine; and
b) the 3′ end of the nopaline synthese gene of Example 9.

pTVEP2, like pTVEP1, is a binery type T-DNA vector containing, within the T-DNA border sequences, three chimeeric genes: PSSU-sfr end PNOS-neo encoding dominant selectable markers for plant transformations; end PTA29-Papain gene which encodes an endopeptidase that will cleave proteins in the tapetum cells, thus leeding to the death of these cells.

Example 15 - Introduction of the chimaeric DNA sequences of Example 14 into tobacco and oilseed rape

As described in Example 11, pTVEP1 and pTVEP2, are each mobilized from E. coli into separate Agrobacterium C58C1 Rif^R carrying pMP90.

The resulting strains, harboring pMP90 with pTVEP1 and pMP90 with pTVEP2, are used to transform tobacco and oilseed rape following the procedures of Examples 11 and 13. Thet the papain genes are not expressed in trensformed herbicide- end kenamycin-resistant calli, shoots and roots is shown by their growth.

The transformed plants are transferred into the greenhouse and grown in soil until they flower. The flowers of both the tobacco and oilseed rape are examined, and phenotypes are observed for the transformed plants that are essentially the same es the phenotype of the transformed tobecco plants described in Example 10. This shows that the TA29 promoter is capable of directing expression of the heterologous papain genes in pTVEP1 and pTVEP2 selectively in tapetum cells of the plants, thereby rendering the plants male-sterile.

Example 16 - Construction of a chimeeric DNA sequence of pTA29 and e gene encoding EcoRI

A plasmid named "pTVE63", shown in Fig.10A, was constructed by assembling the following well known fregments:

1. a vector fragment, including T-DNA border sequences derived from pGSC1701A2 (European petent application 87/115985.1);
2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter, the herbicide-resistence gene sfr and the 3′ end of T-DNA gene 7;

3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene and the 3′ end of the octopine synthase gene;

4. e chimaeric sequence, containing the pTA29 promoter cassette of Example 3, fused in frame with:

e) a gene encoding the EcoRI restriction endonuclease from an E. coli (Green et al (1981) J. Biol. Chem. 256, 2143-2153; Botterman and Zabeau (1985) Gene 37, 229-239) and capable of recognizing end cleeving the target sequence GAATTC on e double stranded DNA; the following modifications were made in the DNA sequence of Green et al (1981) using site directed mutegenesis es described in Example 3:

I. the nucleotides of the ATG initiation codon were replaced by ATGCA, creating e NsiI site et the initiation codon and yielding the following nucleotide sequences:
ATGCA,TCT,AAT...; and

II. the HindII-HindIII fragment of the EcoRI gene cloned in pEcoR12 (Botterman and Zabeau, 1985) was cloned into the pMAC5-8 site directed mutagenesis vector; and

b) the 3′ end of the nopaline synthese gene of Example 9; and

5. a gene encoding an EcoRI methylase under the control of the its natural promoter (Botterman and Zabeau (1985) Gene 37, 229-239) which is capable of inhibiting the activity of EcoRI in E. coli or Agrobacterium, in order to overcome potential leaky expression of the EcoRI gene in microorganisms.

pTVE63 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PSSU-sfr and PNOS-neo which ere marker DNAs under the control of respectively PSSU and PNOS as second promoters; and PTA29-EcoRI gene which is a male-sterility DNA under the control of PTA29 as a first promoter. Expression of the male-sterility DNA under the control of the TA29 promoter in tapetum cells will produce the EcoRI restriction endonuclease which will cleave double stranded DNA at the GAATTC sites (see for review of type II restriction modification systems: Wilson (1988) TIG4 (11), 314-318): of the tapetum cells, thus leeding to the death of these cells.

A plasmid nemed pTVE62, shown in Fig. 10B, was also constructed by essembling the following well known fragments:

1. a vector fragment, including T-DNA border sequences derived from pGSC1701A2;

2. the chimaeric sequence (no. 3) of Example 5, containing the PSSU promoter, the herbicide-resistance gene sfr and the 3′ end of T-DNA gene 7;

3. the chimaeric sequence (no. 4) of Example 5, containing the PNOS promoter, the neo gene and the neo 3′ end of the octopine synthase gene;

4. a chimaeric sequence, containing the pTA29 promoter cassette of Example 3, fused in frame with a gene fragment encoding the transit peptide of the Mn-superoxide dismutase ("Mn-SOD") which is a NcoI-PstI fragment of a HpaI-HindIII fragment from pSOD1 (Bowler et al (1989) Embo J. 8, 31-38); the following modifications were made in the DNA sequence of Bowler et al using site directed mutagenesis as described in Example 3:

I. the AA nucleotides located upstream et position -2 and -1 of the ATG initiation codon were changed to CC nucleotides creating a NcoI site at the initiation codon and yielding the following nucleotide sequences:

```
- CCATGGCACTAC
     NcoI
```

II. the T,TCG,CTC, nucleotides located immediately downstream of the processing site of the transit peptide were changed to C,TGC,AGC, creating a PstI site behind the processing site and yielding the the following nucleotide sequences:

```
                  L   Q   T   F   S   L
CTC,CGC,GGC,      TTG,CAG,ACC,TTT,TCG,CTC
CTC,CGC,GGC,      TTG,CAG,ACC,TTC,TGC,AGC...
                                  PstI
```

in which the arrow indicetes the processing site of the transit peptide sequence and the upper line the aminoacid sequence corresponding with the Mn-SOD coding sequence; the NcoI-PstI fragment was also fused in frame with a gene encoding the EcoRI restriction endonuclease from E. coli (Greene et al (1981) J. Biol. Chem. 256, 2143-2153; Botterman and Zabeau (1985) Gene 37, 229-239) and capable of recognition and cleavage of the target sequence GAATTC on e double stranded DNA, as found in pTVE63; and

b) the 3′ end of the nopaline synthase gene of Example 9; and

5. a gene encoding the EcoRI methylase under the control of its netural promoter (Botterman and Zabeau, 1985) which is capable of inhibiting the ectivity of EcoRI in E. coli or Agrobacterium, in order to

overcome potential leaky expression of the EcoRI gene in microorganisms, this gene being inserted into the vector fragment outside the border sequences.

pTVE62 is e binary type T-DNA vector containing, within the border sequences, three chimeric sequences: PSSU-sfr and PNOS-NPTII which are marker DNAs under the control of respectively PSSU and PNOS as second promoters; and pTA29-transit peptide-EcoRI endonuclease gene which is a male-sterility DNA having PTA29 as a first promoter and e transit peptide-encoding sequence between them. Expression of the male-sterility DNA under the control of the TA29 promoter in tepetum cells will produce e restriction endonuclease which will be targeted into the mitochondria of the tepetum cells and cleave the double stranded DNA et the CAATTC sites in such cells. This will leed to the death of these cells.

Example 17 - Introduction of the chimaeric DNA sequences of Example 16 into tobacco and oilseed rape

As described in Examples 11 and 15, pTVE62 and pTVE63, were mobilized from E. coli into Agrobacterium C58C1 Rif^R carrying pMP90. The resulting strains, harboring pTVE62 with pMP90 and pTVE62 (with pMP90, were used to transform tobacco and are used to transform oilseed rape following the procedures described in Examples 11 and 13. That the EcoRI endonuclease genes were not expressed in transformed herbicide- and kanamycin-resistant calli, shoots and roots is shown by their growth.

The transformed plants are transferred into the greenhouse and grown in soil until they flower. The flowere of both the tobacco and oilseed rape are examined, and phenotypes are observed for the transformed plants that are essentially the same as of the transformed tobacco plants described in Example 10. This shows that the TA29 promoter is capable of directing expression of the heterologous EcoRI endonuclease gene selectively in the tapetum cells of the plants transformed with pTVE62 and pTVE63, thereby rendering the plants male-sterile.

Needless to say, this invention is not limited to the transformation of any specific plant(s). The invention relates to any plant, the nuclear genome of which can be transformed with a male-sterility DNA under the control of a first promoter that can direct expression of the male-sterility DNA selectively in the plant's stamen cells, whereby the plant can be both self-pollineted and cross-pollineted. For example, this invention reletes to plants such as potato, tomeio, oilseed rape, alfalfa, sunflower, cotton, celery, onion, corn, soybean, tobacco, brassice vegetables and sugarbeet.

Also, this invention is not limited to the specific plesmids and vectors described in the foregoing Exemples, but rather encompasses any plasmids and vectors containing the male-sterility DNA under the control of the first promoter.

Furthermore, this invention is not limited to the specific promoters described in the foregoing Examples, such es the TA29 promoter, but rather encompass any DNA sequence encoding a promoter capable of directing expression of the male-sterility DNA selectively in stamen cells. In this regard, this invention encompasses the DNA sequence of the TA29 promoter of Fig. 3A, as well as any equivalent DNA sequences, such as that of the TA13 promoter of Fig. 3B and the TA 26 promoter of Fig. 3C, which can be used to control the expression of the male-sterility DNA selectively in tepetum cells of e plant. Indeed, it is believed that the DNA sequences of fhe TA29, TA26 and TA13 promoters can be modified by: 1) replacing some codons with others that code either for the same amino acids or for other amino acids; and/or 2) deleting or adding some codons; provided that such modifications do not substantially alter the properties of the encoded promoter for controlling tapetum-specific expression of e male-sterility.

In addition, this invention is not limited to the specific male-sterility DNAs described in the foregoing Examples but rather encompasses eny DNA sequence encoding a first RNA, protein or polypeptide which disturbs significantly the metabolism functioning and/or development of a stamen cell in which it is produced, under the control of the first promoter.

Also, this invention is not limited to the specific marker DNAs described in the foregoing Examples but rather encompasses any DNA sequence encoding e second RNA, protein or polypeptide which confers on at least a specific plant tissue or specific plant cells, in which such DNA sequence is expressed, e distinctive trait compared to such a specific plant tissue or specific plant cells in which such DNA sequence is not expressed.

Claims

1. A cell of a plant, the nuclear genome of which is transformed with a foreing DNA sequence, preferably a foreing chimaeric DNA sequence, characterized by:

(e) e male-sterility DNA, encoding a first RNA, protein or polypeptide which, when produced or overproduced in e stamen cell of said plant, disturbs significantly the metebolism, functioning and/or development of said stamen cell; and

(b) e first promoter capable of directing expression of said male-sterility DNA selectively in stamen cells of said plant, preferably in anther, pollen and/or filament cells, particularly in tapetum and/or anther epidermal cells; said male-sterility DNA being in the same transcriptional unit as, and under the control of, said first promoter.

2. The cell of claim 1, wherein said foreign DNA sequence also comprises, preferably in the seme genetic locus as said male-sterility DNA:

(c) a marker DNA encoding a second RNA, protein or polypeptide which, when present at least in a

specific tissue or in et leest specific cells of said plant, renders said plant easily separable from other plants which do not contain said second RNA, protein or polypeptide et least in said specific tissue or specific cells; and

(d) a second promoter capable of directing expression of said marker DNA et least in said specific tissue or specific cells; said marker DNA being in the same transcriptional unit es, end under the control of, said second promoter.

3. The cell of claim 1 or 2, wherein said foreign DNA sequence further comprises:

(e) e first DNA encoding a transit peptide capable of transporting said first protein or polypeptide into a chloroplast or mitochondria of said stamen cell; said first DNA being in the same transcriptional unit as said male-sterility DNA and said first promoter and between said male-sterility DNA and said first promoter; and/or

f) a second DNA encoding a transit peptide capable of transporting said second protein or polypeptide into a chloroplast or mitochondria of et least said specific tissue or specific cells; said second DNA being in the same transcriptional unit as said marker DNA and said second promoter and between said marker DNA end said second promoter.

4. The cell of anyone of claims 1-3, wherein said male-sterility DNA encodes: an RNase, particularly RNase T1 or Barnese; a DNase, especially an endonuclease, particularly EcoRI; e protease, especially a papain, particularly papain Zymogen or papein active protein; a glucanase; e lipase, particularly phospholipase A_2; e lipid peroxidase; e cell wall inhibitor; e bacterial toxin; or a ribozyme; particularly the ribozyme against mRNA encoded by the TA29 gene, the TA26 gene or the TA13 gene; or is an anti-sense DNA, particularly the antisense DNA of the TA29 gene, the TA26 gene or the TA13 gene.

5. The cell of anyone of claims 1-4, wherein said male-sterility DNA encodes an enzyme which catalyzes the synthesis of a phytohormone, particularly an enzyme encoded by gene 1, gene 2 or gene 4 of Agrobecterium T-DNA.

6. The cell of enyone of claims 2-5, wherein said marker DNA is: an herbicide resistence gene, particularly an sfr or sfrv gene; a gene encoding e modified target enzyme for an herbicide having lower effinity for the herbicide, particularly e modified 5-enolpyruvylshikimate-3 phosphate synthese as a target for glyphosate or a modified glutemine synthetase es e target for a glutamine synthetase inhibitor such as phosphinotricine; a gene encoding a protein or a polypeptide conferring a color to at least said specific tissue or specific cells, particulairy the gene A1 or the GUS gene; a gene encoding a protein or a polypeptide conferring a stress tolerance to said plant, particularly the gene encoding Mn-superoxide dismutase; or e gene encoding a protein or a polypeptide conferring a disease or pest resistance, particularly a gene encoding e Bacillus thuringiensis endotoxin that confers insect resistance or e gene encoding a bactericidal peptide that confers e bacteriel resistance.

7. The cell of anyone of claims 1-6, wherein said first promoter is PTA29, PTA26, PTA13 or a promoter of a DNA coding for a tepetum-specific mRNA hybridizable to TA29, TA26 or TA13.

8. The cell of anyone of claims 2-7, wherein said second promoter is: a constitutive promoter, particularly a 35S promoter, a 35S'3 promoter, a PNOS promoter or a POCS promoter; a wound-inducible promoter, particularly e TR1' or TR2' promoter; e promoter which directs gene expression selectively in plent tissue heving photosynthetic activity, particularly en SSU promoter; or a promoter which directs gene expression selectively in leef cells, petal cells or seed cells, particularly seed coat cells.

9. A vector suitable for transforming e cell of a plant, perticularly a plant capable of being infected with Agrobecterium, comprising seid foreign DNA sequence of anyone of claims 1-8, particularly pTTM4, pTTM6, pTTM6A⁻, pTTM8, pTVEP1, pTVEP2, pTVE62 or pTVE63.

10. A process for producing e male-sterile plent end reproduction materiel of said plant, having said foreign DNA sequence of anyone of claims 2-8 stably integrated into the nuclear genome of their cells, whereby said male-sterility DNA is capeble of being expressed selectively in stamen cells of said plant to produce said first RNA, protein or polypeptide in said stamen cells and whereby said merker DNA can be expressed in at least said specific tissue or specific cells of said plant to render said plant separable from non-transformed plants, characterized by the non-biological steps of: e) transforming e cell of said plant by introducing said foreign DNA sequence into the nuclear genome of said cell; and then b) regenerating said plant end reproduction meterials from said cell.

11. A plant cell culture, containing the plant cell of anyone of claims 1-8.

12. A plant, particularly corn, potato, tomato, oilseed rape, alfafa, sunflower, cotton, celery, onion, clover, soybean, tobacco, bressica vegetebles aor sugarbeet, containing the plant cell or anyone of claims 1-8.

13. A seed of a plant of claim 12.

14. A process for producing a hybrid seed, characterized by the steps of: a) cross-pollinating i) a male-sterile plant that contains the foreign DNA sequence of anyone of claims 2-8, including both said second promoter and said marker DNA, especially said marker DNA conferring a resistence to an herbicide, particularly an sfr or srfv gene, stably integrated in the nuclear genome of the cells of said male-sterile plent, with II) a homozygous male-fertile plant without said merker DNA, especially without said marker DNA conferring said herbicide resistance; and then b) separating said male-fertile plant from said male-sterile plant by taking advantage of the absence of expression of said marker DNA et least in said specific tissue or specific cells of said male-fertile plant.

15. The process for producing e hybrid seed of claim 14, wherein said male-sterile plant conteins at least

two different marker DNAs stably integrated into the nuclear genome of its cells and said male-fertile plant contains one, but not the other, of said two marker DNAs; and wherein said male-fertile plant is separated from said male-sterile plant by taking advantage of the absence of expression of said other maker DNA at least in said specific tissue or specific cells of said male-fertile plant; said other marker DNA preferably conferring a resistance to an herbicide.

16. The hybrid seed obtained by the process of claim 14 or claim 15.

17. A hybrid plant obtained by growing the hybrid seed of claim 16.

18. The first promoter of claim 7, particularly PTA29, PTA26 or PTA13.

19. A foreign chimaeric DNA sequence of anyone of claims 1-8, wherein said male-sterility DNA is not naturally found under the control of the first promoter and/or is not naturally found in the same genetic locus as said marker DNA.

20. In a process for producing plant and reproduction material, such as seeds, of said plants including a foreign genetic material stably integrated in nuclear genome thereof and capable of being expressed therein as an RNA, protein or polypeptide, comprising the non-biological steps of: a) producing transformed plants cells or plant tissue including said foreign genetic material from starting plant cells or plant tissue not expressing said RNA, protein or polypeptide, b) producing regenerated plants or reproduction material of said plants or both from said transformed plant cells or plant tissue including said foreign genetic material, and c) optionally, biologically replicating said regenerated plants or reproduction material or both; wherein said step of producing said transformed plant cells or plant tissue including said foreign genetic material is characterized by: transforming the nuclear genome of said starting plant cells or plant tissue with a foreign DNA sequence of anyone of claims 1-8, as well as regulatory elements which are capable of enabling the expression of said foreign DNA sequence in said plant cells or plant tissue, to cause the stable integration of said foreign DNA sequence in transformed plant cells or plant tissue, as well as in plants and reproduction material produced therefrom throughout subsequent generations.

FIG. 1

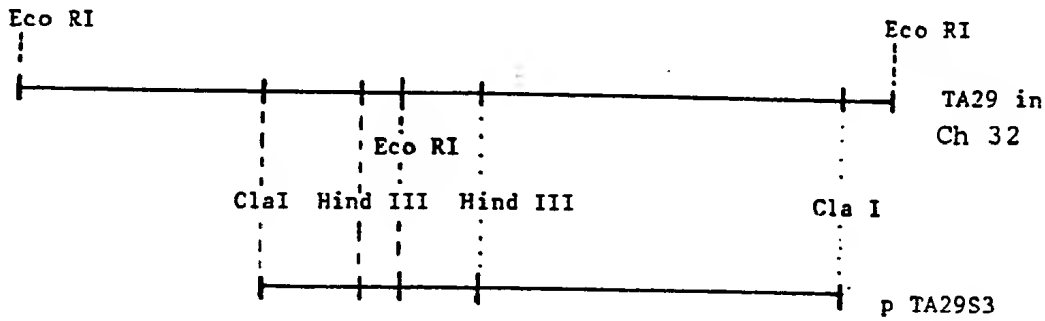

Eco RI
Eco RI
TA29 in
Ch 32
Eco RI
ClaI
Hind III
Hind III
Cla I
p TA29S3

Fig. 2

```
                                                                    27
CAA TCC GCT AGA CTA TAC CGT TGC AAG CCA GGG CCA AAT ATG TGT GAC AGT AAA                54
Q  S  A  R  L  Y  R  C  K  P  G  P  N  M  C  D  S  K

                                                                    81
GAC TGT AAT GAG CTT CTC CTA CAC TTT GTT TTC CCA ATG CAA GAC AAA CAT GAC              108
D  C  N  E  L  L  L  H  F  V  F  P  M  Q  D  K  H  D

                                                                   135
AAT AAA CAA GAA CAT CTA AGA TAT GGA GGA CGC CGA GGT ATA GGT CTC ACT GTG              162
N  K  Q  E  H  L  R  Y  G  G  R  R  G  I  G  L  T  V

                                                                   189
GGA GGA GTT GGC GGT TTT GGA ATT GGT TTT GGT GCT TGG GGT GGT GGT GGT GGC              216
G  G  V  G  G  F  G  I  G  F  G  A  W  G  G  G  G  G

                                                                   243
GGA GGA GGT GGT GGT TCT GAT GCC CCT GGT TGT AGT AAC GAT GGC TGT GAC CCT              270
G  G  G  G  G  S  D  A  P  G  C  S  N  D  G  C  D  P

                                                                   297
GGT TTT GGC TGT CCC CCG GGC TGT GGT TAT GCA TGT CCT GCC AAC AAT CCT AGT              324
G  F  G  C  P  P  G  C  G  Y  A  C  P  A  N  N  P  S

                                                                   351
GGA GGA ATA ACT GAA TTC CAT ATC TCA GGA TTG TTG GCA
G  G  I  T  E  F  H  I  S  G  L  L  A
```

Figure 3A

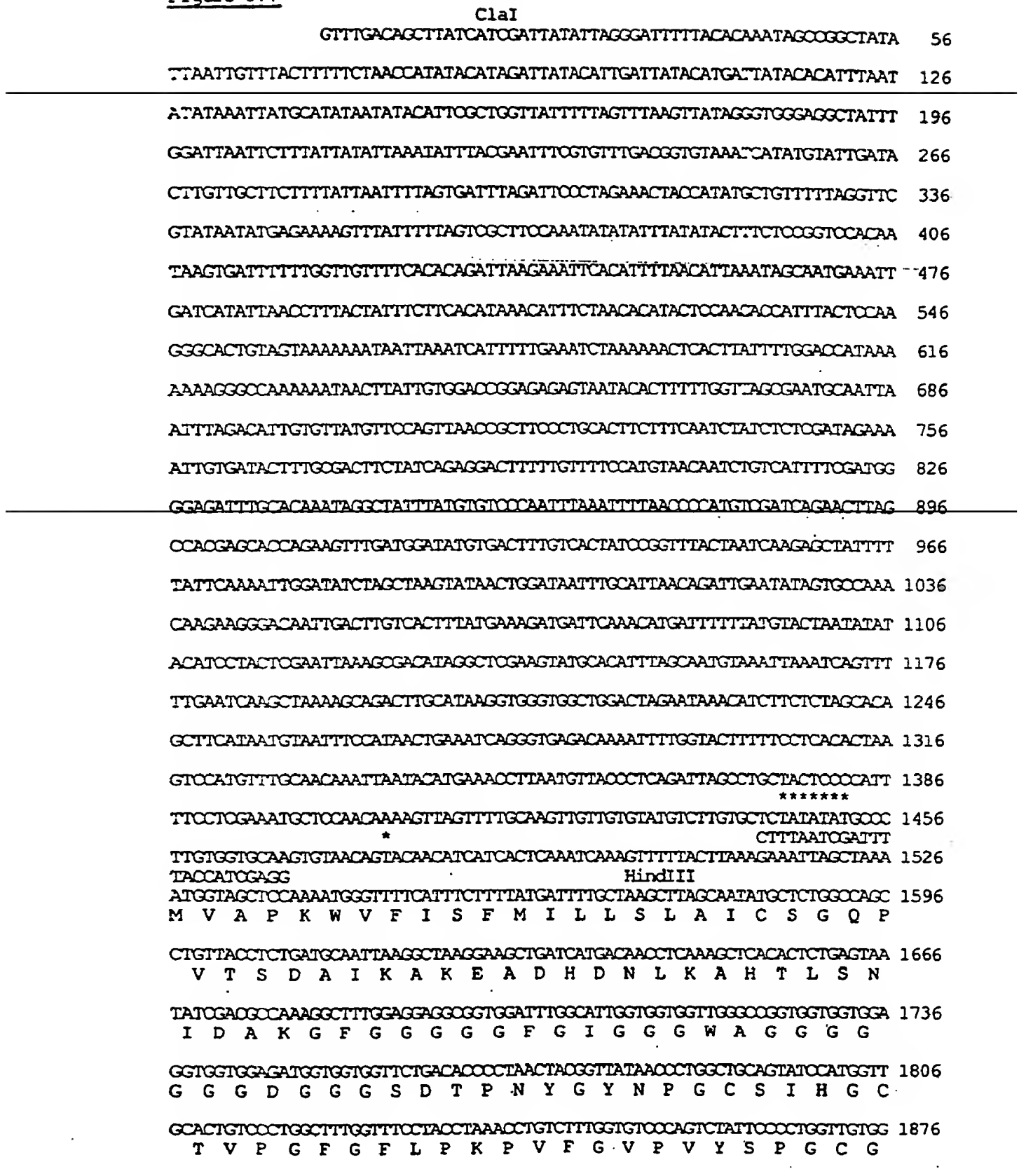

ClaI
GTTTGACAGCTTATCATCGATTATATTAGGGATTTTTACACAAATAGCCGGCTATA 56
TTAATTGTTTACTTTTTCTAACCATATACATAGATTATACATTGATTATACATGATTATACACATTTAAT 126
ATATAAATTATGCATATAATATACATTCGCTGGTTATTTTTAGTTTAAGTTATAGGGTGGGAGGCTATTT 196
GGATTAATTCTTTATTATATTAAATATTTACGAATTTCGTGTTTGACGGTGTAAACCATATGTATTGATA 266
CTTGTTGCTTCTTTTATTAATTTTAGTGATTTAGATTCCCTAGAAACTACCATATGCTGTTTTTAGGTTC 336
GTATAATATGAGAAAAGTTTATTTTTAGTCGCTTCCAAATATATATTTATATACTTTCTCCGGTCCACAA 406
TAAGTGATTTTTTGGTTGTTTTCACACAGATTAAGAAATTCACATTTTAACATTAAATAGCAATGAAATT 476
GATCATATTAACCTTTACTATTTCTTCACATAAACATTTCTAACACATACTCCAACACCATTTACTCCAA 546
GGGCACTGTAGTAAAAAAAATAATTAAATCATTTTTGAAATCTAAAAAACTCACTTATTTTGGACCATAAA 616
AAAAGGGCCAAAAAATAACTTATTGTGGACCGGAGAGAGTAATACACTTTTTGGTTAGCGAATGCAATTA 686
ATTTAGACATTGTGTTATGTTCCAGTTAACCGCTTCCCTGCACTTCTTTCAATCTATCTCTCGATAGAAA 756
ATTGTGATACTTTGCGACTTCTATCAGAGGACTTTTTGTTTTCCATGTAACAATCTGTCATTTTCGATGG 826
GGAGATTTGCACAAATAGGCTATTTATGTGTCCCAATTTAAATTTTAACCCCATGTCGATCAGAACTTAG 896
CCACGAGCACCAGAAGTTTGATGGATATGTGACTTTGTCACTATCCGGTTTACTAATCAAGAGCTATTTT 966
TATTCAAAATTGGATATCTAGCTAAGTATAACTGGATAATTTGCATTAACAGATTGAATATAGTGCCAAA 1036
CAAGAAGGGACAATTGACTTGTCACTTTATGAAAGATGATTCAAACATGATTTTTTATGTACTAATATAT 1106
ACATCCTACTCGAATTAAAGCGACATAGGCTCGAAGTATGCACATTTAGCAATGTAAATTAAATCAGTTT 1176
TTGAATCAAGCTAAAAGCAGACTTGCATAAGGTGGGTGGCTGGACTAGAATAAACATCTTCTCTAGCACA 1246
GCTTCATAATGTAATTTCCATAACTGAAATCAGGGTGAGACAAAATTTTGGTACTTTTTCCTCACACTAA 1316
GTCCATGTTTGCAACAAATTAATACATGAAACCTTAATGTTACCCTCAGATTAGCCTGCTACTCCCCATT 1386
****
TTCCTCGAAATGCTCCAACAAAAGTTAGTTTTGCAAGTTGTTGTGTATGTCTTGTGCTCTATATATGCCC 1456
* CTTTAATCGATTT
TTGTGGTGCAAGTGTAACAGTACAACATCATCACTCAAATCAAAGTTTTTACTTAAAGAAATTAGCTAAA 1526
TACCATCGAGG HindIII
ATGGTAGCTCCAAAATGGGTTTTCATTTCTTTTATGATTTTGCTAAGCTTAGCAATATGCTCTGGCCAGC 1596
M V A P K W V F I S F M I L L S L A I C S G Q P
CTGTTACCTCTGATGCAATTAAGGCTAAGGAAGCTGATCATGACAACCTCAAAGCTCACACTCTGAGTAA 1666
V T S D A I K A K E A D H D N L K A H T L S N
TATCGACGCCAAAGGCTTTGGAGGAGGCGGTGGATTTGGCATTGGTGGTGGTTGGGCCGGTGGTGGTGGA 1736
I D A K G F G G G G G F G I G G G W A G G G G
GGTGGTGGAGATGGTGGTGGTTCTGACACCCCTAACTACGGTTATAACCCTGGCTGCAGTATCCATGGTT 1806
G G G D G G G S D T P N Y G Y N P G C S I H G C
GCACTGTCCCTGGCTTTGGTTTCCTACCTAAACCTGTCTTTGGTGTCCCAGTCTATTCCCCTGGTTGTGG 1876
T V P G F G F L P K P V F G V P V Y S P G C G

Figure 3 A (continued)

cDNA clone TA29 --> ACAATCC

CTATGTGTGTCCGGCCGATATTCCTACTGGAGGAATGACTGAATCCAAAATCACAGGAATATCACAATCC 1946
Y V C P A D I P T G G M T E S K I T G I S Q S

GCTAGACTATACCGTTGCAAGCCAGGGCCAAATATGTGTGACAGTAAAGACTGTAATGAGCTTCTCCTAC
GCTAGACTATACCGTTGCAAGCCAGGGCCAAATATGTGTGACAGTAAAGACTGTAATGAGCTTCTCCTAC 2016
A R L Y R C K P G P N M C D S K D C N E L L L H

ACTTTGTTTTCCCAATGCAAGACAAACATGACAATAAACAAGAACATCTAAGATATGGAGGACGCCGAGG
ACTTTGTTTTCCCAATGCAAGACAAACATGACAATAAACAAGAACATCTAAGATATGGAGGACGCCGAGG 2086
F V F P M Q D K H D N K Q E H L R Y G G R R G

TATAGGTCTCACTGTGGGAGGAGTTGGCGGTTTTGGAATTGGTTTTGGTGCTTGGGGTGGTGGTGGTGGC
TATAGGTCTCACTGTGGGAGGAGTTGGCGGTTTTGGAATTGGTTTTGGTGCTTGGGGTGGTGGTGGTGGC 2156
I G L T V G G V G G F G I G F G A W G G G G G

GGAGGAGGTGGTGGTTCTGATGCCCCTGGTTGTAGTAACGATGGCTGTGACCCTGGTTTTGGCTGTCCCC
GGAGGAGGTGGTGGTTCTGATGCCCCTGGTTGTAGTAACGATGGCTGTGACCCTGGTTTTGGCTGTCCCC 2226
G G G G G S D A P G C S N D G C D P G F G C P P

EcoRI

CGGGCTGTGGTTATGCATGTCCTGCCAACAATCCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATT
CGGGCTGTGGTTATGCATGTCCTGCCAACAATCCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATT 2296
G C G Y A C P A N N P S G G I T E F H I S G L

<-- end cDNA clone TA29

ATCACGATTCGATGGACCTTACAGATGTAGGCCAGATATGTGTGAAAGTGAAGATTGTAATGAACTTCTT 2366
S R F D G P Y R C R P D M C E S E D C N E L L

CTACACTTTGTTTCTCCAATGCAACACAAACATGAGAACCGACATGATCATATAGTAGAAAGGAGTGATG 2436
L H F V S P M Q H K H E N R H D H I V E R S D E

AGGAGGAAGCGCATCATCAGTCAAAGCAGCATAAAGACGAAGACATCATAAACTAGGCTCTCCCACAAAC 2506
E E A H H Q S K Q H K D E D I I N *

CAAAAAAAAAGGAACTATATATGTAGCTTCAGCCAAAAAACTGTATACACTGTCTAAGAATACTCACTTC 2576

CAACGAACTTAAATAAAACTAGTTTACAGTGGATTGGGATATAATCAGTTGGACAATTTGCTAAACCTCC 2646

TCATGCACTGTAAAAATAGACTTGCTACTAGTATTTGGAATATAATGCTGAATATATTTGTTGTTACTTT 2716

GCCTAATGTCAATCAGCATTCAGCAATTTCTCTGTAGTTAGAAAATGAAAGGAAGAATCAGGAAACTCAT 2786

ATTTAAAGGATGAAATAATTTAAAGATCGCGAAGCAGTCACAATTTAATAGTACCAGGAAAATAATCTAT 2856

AGGAATCACAGAACTTTTTGATTTATCAAATTAAGGAAGCAAACTGGGAAAATGTGAAATGAATGAACAT 2926

AATGCTGAAAGCTATTGATCAGATGATTGGATTGATTTCGTAGGAGCAACATATGATTTAAGATTATTTC 2996

AACAAGATGGCCATAAAGTAGCATATCATTTGTAATTTAACATTATTACACTCAAACTCAGGAAGATTGT 3066

CAATTTACCCTCAAAACAAAGTTTTAAGCCTTCAGTCTCCTTCAACCACAGTGGCACCTGCCCAATTGGC 3136

AGCACTTCCCCGGCGTGGAATGCTGTGGAGTTTGGTGTACAAATCCACCTGGAAAATCACAGCATTGATG 3206

HindIII

TTTCCTTCATCATCTTCGCGTGCAATTGCTTTTACTTTGTGCAGTGGATGATCAAAGCTT 3266

Figure 3 B

```
  1 aaagtctttacttaaagaaattagctaaaatggtagctccaaaatggtctttcatttcttttatgattttgc

 73 taagcttagcaatatgctctggccagcctgttacctttgatgcaattaaggctaaggaagctgatcatgaca

145 acctcaaagctcacactctaagtaatatcgacaccaaaggctttggaggaggcggtggatttggcattggtg

217 gtgtttgggccggaggtggtggtggtggttctgacgccctaactacggttataaccctggctgcagta

289 tccgtggttgcactgtccctggctttggtttcctacctaatcctggttttggtgttccagtctattcccctg

361 gttgtggctatgtgtgtccagccgatatttctgctgaaggaatgactgaatccaaaatcacaggaatatcAg

  1                                                                      |
                                                                         Ac

433 AATCgGCTAGACcATAtOGaTGCAgGOCtGGGgCAAATATGTGTGgCAGTAAAGAtTGTAATGAGCTTCTCC
    |||| |||||||| ||| || |||| ||| ||| |||||||||||| ||||||||| ||||||||||||||||
  8 AATCcGCTAGACtATAcOGtTGCAaGOCaGGGcCAAATATGTGTGaCAGTAAAGAcTGTAATGAGCTTCTCC

505 TACACTTTGTTTTOOCcATGCAAGACAAACATGAgAATAAACAAGAAtATCTAAGATATGGAGGACGtCGAG
    |||||||||||||||| |||||||||||||||| |||||||||||| |||||||||||||||||||| ||||
 75 TACACTTTGTTTTOOCaATGCAAGACAAACATGAcAATAAACAAGAAcATCTAAGATATGGAGGACGcCGAG

577 GTATAGGTCTCAgTGTGaGtGaAtcTaGtGGTTTTGGAATTGGTTTTGGTGCTcgggGtGGTGGTGGTGGcG
    |||||||||||| |||| | | |  | | ||||||||||||||||||||||||    | ||||||||||| |
147 GTATAGGTCTCAcTGTGgGaGgAgtTgGcGGTTTTGGAATTGGTTTTGGTGCT   tGgGGTGGTGGTGGtG

649 GOGGAGGAGGgGGTGaTTCTaATGOOOCTGGcTtTgaTAtCoooGGaTtTaAOOOcGGcTTTGGCTGTOOOCt
    |||||||||| |||| |||| |||||||||| | |  || |   || | | |||| || ||||||||||||
216 GOGGAGGAGGtGGTGgTTCTgATGOOOCTGGtTgTagTAaCgatGGcTgTgAOOOCtGGtTTTGGCTGTOOOCc

721 CGGGCTGTGGTTATGCATGTCCTGOCAACAATOCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATTaT
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
288 CGGGCTGTGGTTATGCATGTCCTGOCAACAATOCTAGTGGAGGAATAACTGAATTCCATATCTCAGGATTgT

793 cacgAaacaatggaccttacagatgtaggccagatatgtgtgagagtgaagattgtaatgaacttctactac
      |
360 tggcAg

865 actttgtttctccaaagcaacacaaacacgagaaccgacatgatcatacagtagaaagaaatgaagaggagg

937 aagctcatcatcagtcaaagcatcataaagacgaagacatcataaactag
```

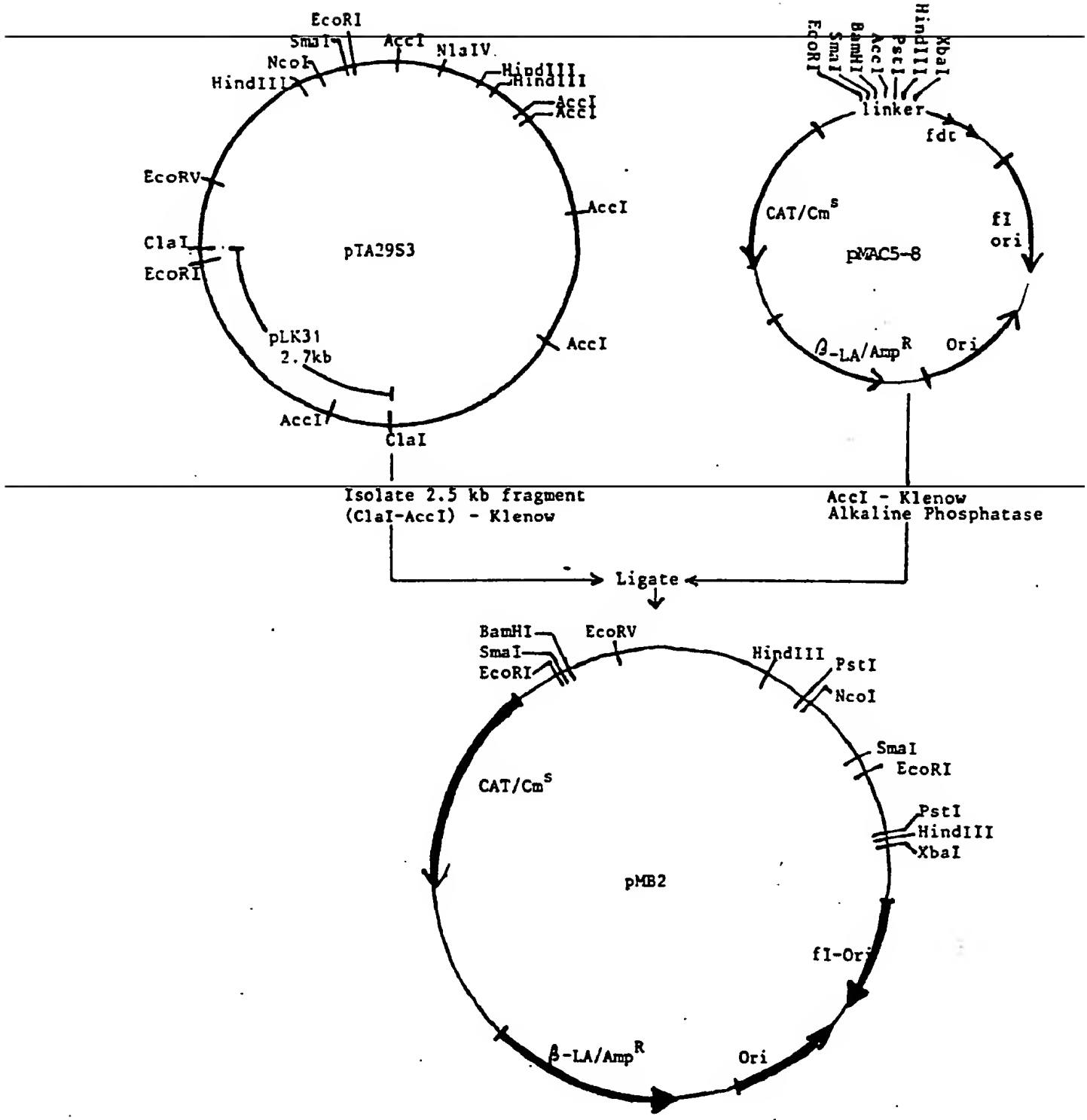
SEQUENCE OF cDNA TA26

```
        10         20         30         40         50         60         70
CGCAGGGGGG GGGGGGGGGG GGGGGGCAAA GATGTCAATA ACTTCCAAGT TCTTGCTAGT TATGTCCCTA

        80         90        100        110        120        130        140
GGACTAATAG TTTTTACCAC ATTTTCACTT GCTGATCAAC ACTACCAATC TACCAAACAT GAGCTTGGAC

       150        160        170        180        190        200        210
GTTCTGATAC TAATCAGCTA AACATGAATG GTTACTTAGC CATGGAACCA GCACCACCAG ACCTTGAGCA

       220        230        240        250        260        270        280
AGAAGGGCAT ATGTGGCGCT TGAACGACGA CTCGATCGCC ATGGAACCAG CACCACCAGA CCTTGAGCAA

       290        300        310        320        330        340        350
GAAGTGCATA TGTGGCGCTT GAACGACGAC TCGATCGCCA TGGAACCAGC ACCAAGGTTT GAGCTAGAAG

       360        370        380        390        400        410        420
GGCAGAAGCA ACATGAGCAT GAGTCACACT TGAGGCTAGT AACTTAGAAA ACATGATTAG CACCAGAATA

       430        440        450        460        470        480        490
GAATTAACTT GGAAGATGGT GGATTATTGT ACTATAGTCC CTTATTCTAA GTTGTGGATC AATAATAAAG

       500        510        520        530        540        550        560
CTCCATTGTC CTAAATTTCC ATCTGAGTTA AATTATCACC TTATAATTAA GTACCCCCCC CCCCCCCCCC
```

C

FIGURE 3C

Fig. 4A


EcoRI
SmaI
AccI
NlaIV
NcoI
HindIII
HindIII
HindIII
AccI
AccI
EcoRV
AccI
ClaI
EcoRI
pTA29S3
pLK31
2.7kb
AccI
AccI
ClaI
EcoRI
SmaI
BamHI
AccI
PstI
HindIII
XbaI
linker
fdt
CAT/Cm^S
fI
ori
pMAC5-8
β-LA/Amp^R
Ori
Isolate 2.5 kb fragment
(ClaI-AccI) - Klenow
AccI - Klenow
Alkaline Phosphatase
Ligate
BamHI
SmaI
EcoRI
EcoRV
HindIII
PstI
NcoI
SmaI
EcoRI
CAT/Cm^S
PstI
HindIII
XbaI
pMB2
fI-Ori
β-LA/Amp^R
Ori

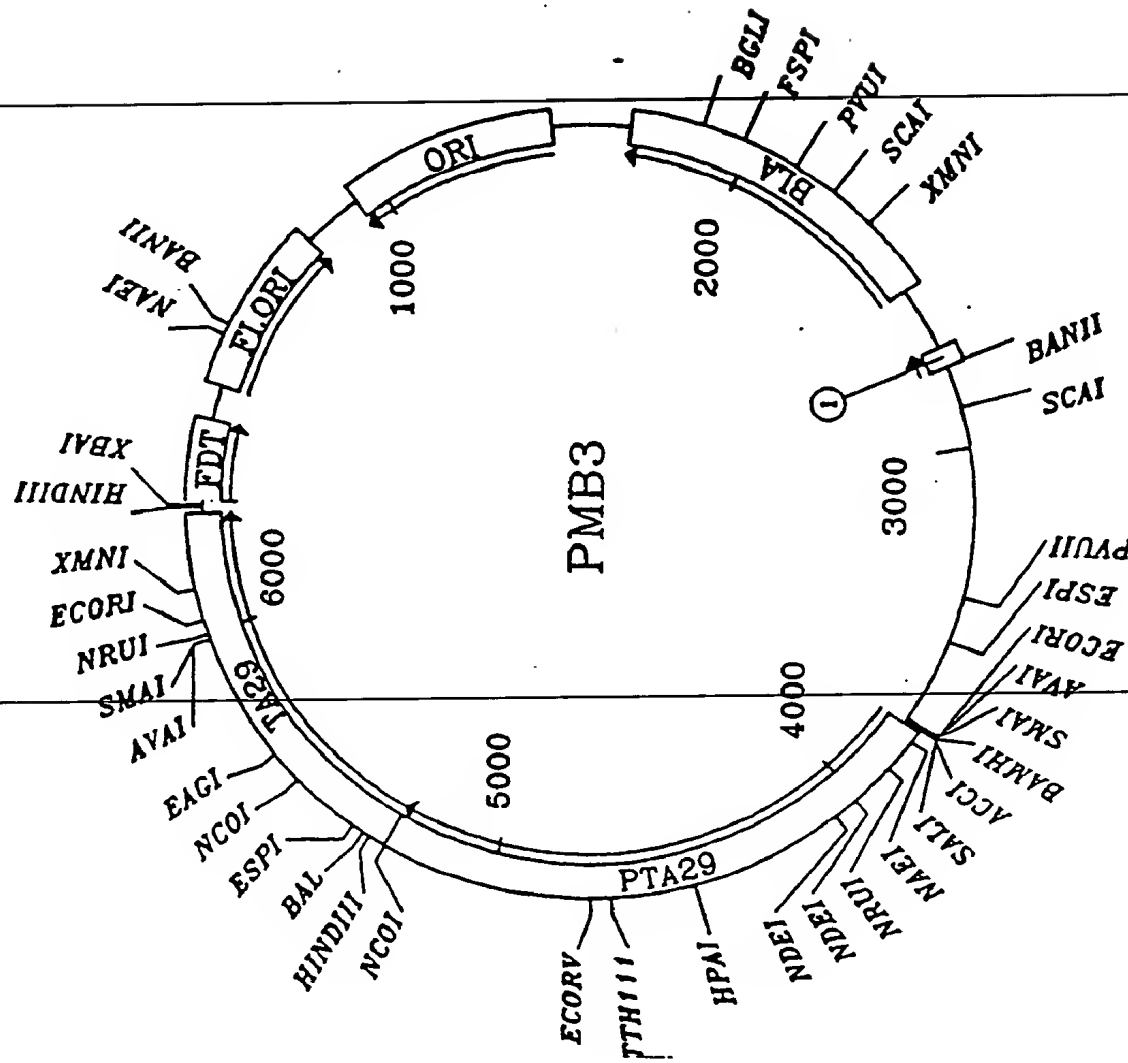


FIG.4 B

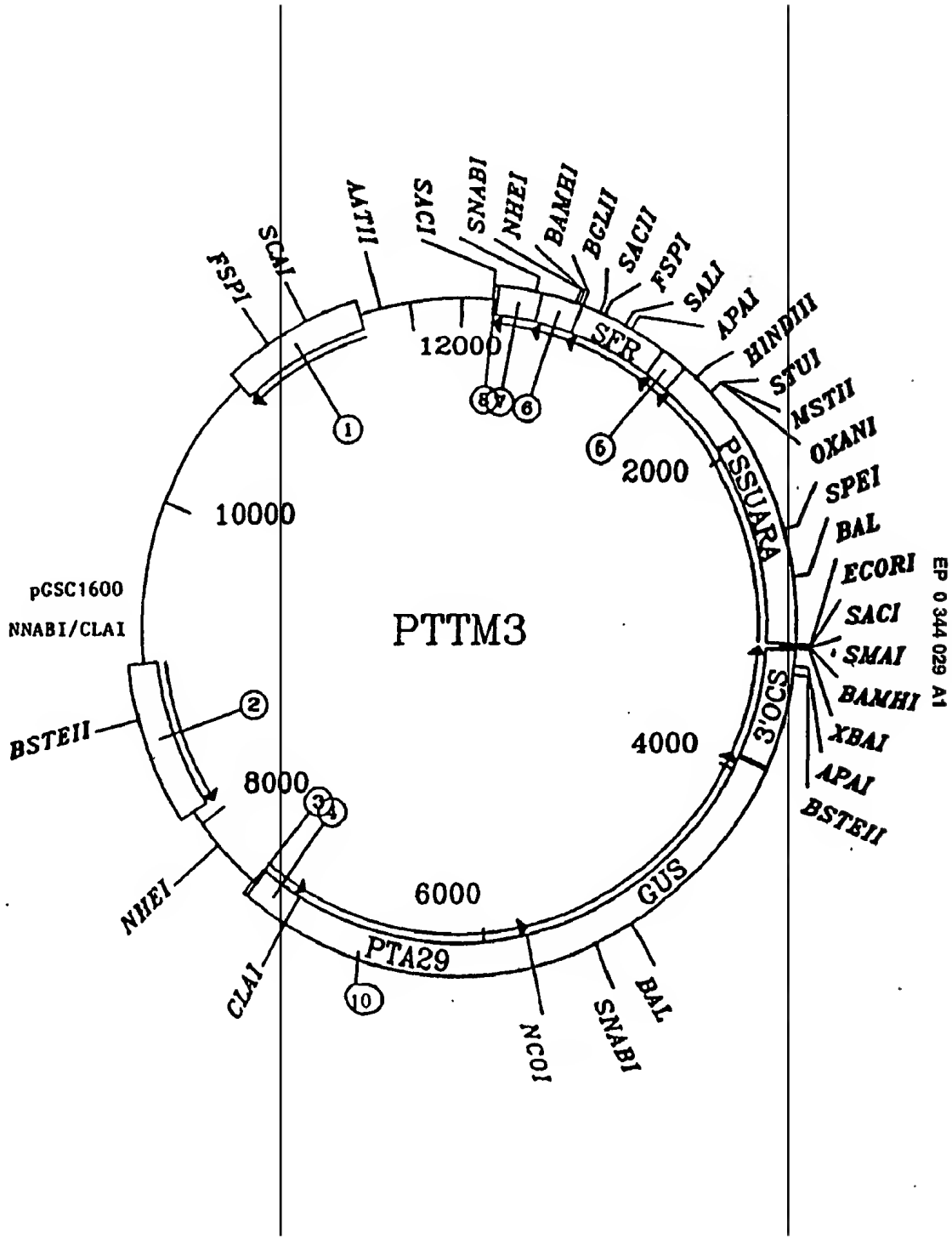
LEGEND

① TT

FIG. 5

LEGEND

1. BETA-LACTAMASE
2. SM-SP-AD.-TRANSF.
3. L.B.
4. T-DNA
5. TP
6. 3'END T7
7. T-DNA
8. R.B.
10. PTA29S3/pMB3



EP 0 344 029 A1

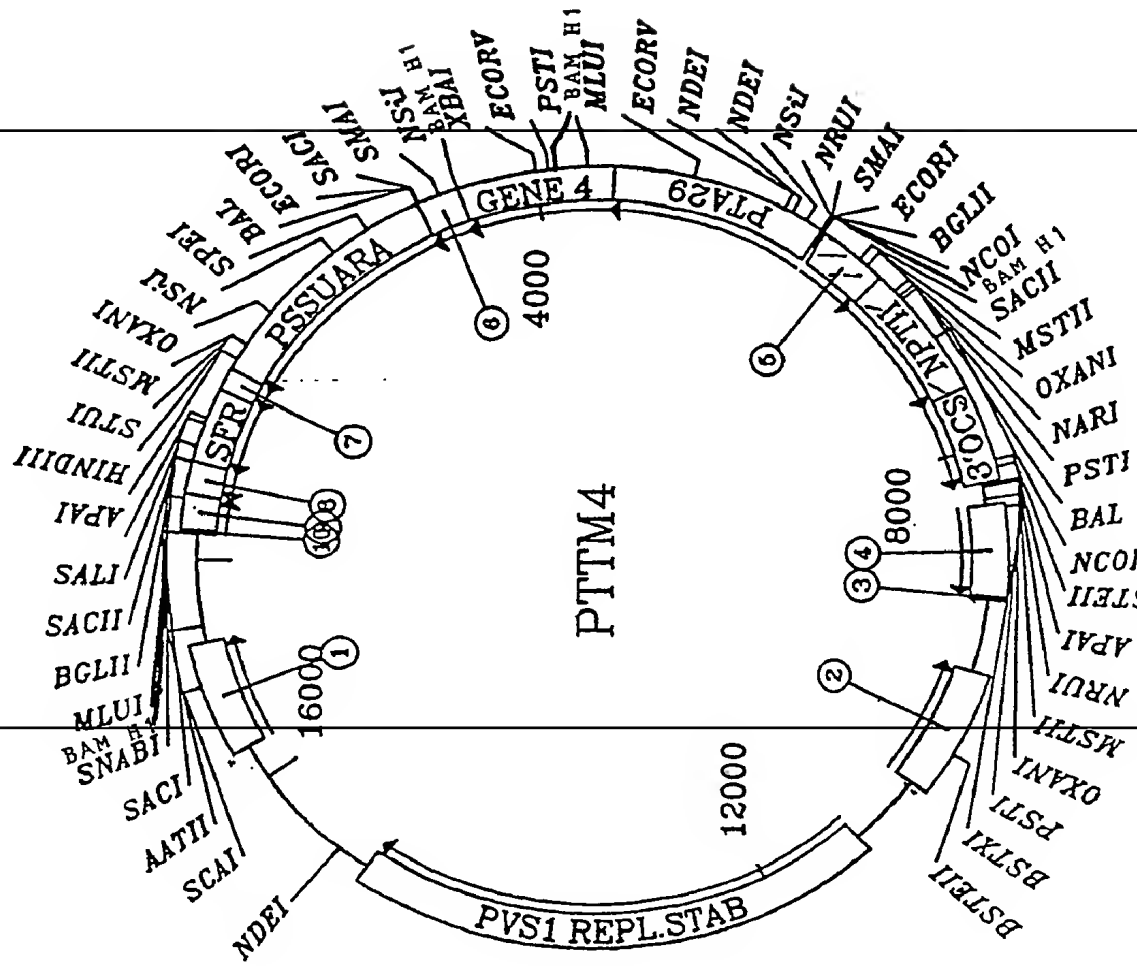


FIG. 6

LEGEND

① BETA-LACTAMASE
② SM-SP-AD TRANSF.
③ LB
④ T-DNA
⑤ PNOS
⑥ 3'NOS
⑦ TP
⑧ 3'END T7
⑨ T-DNA
⑩ RB

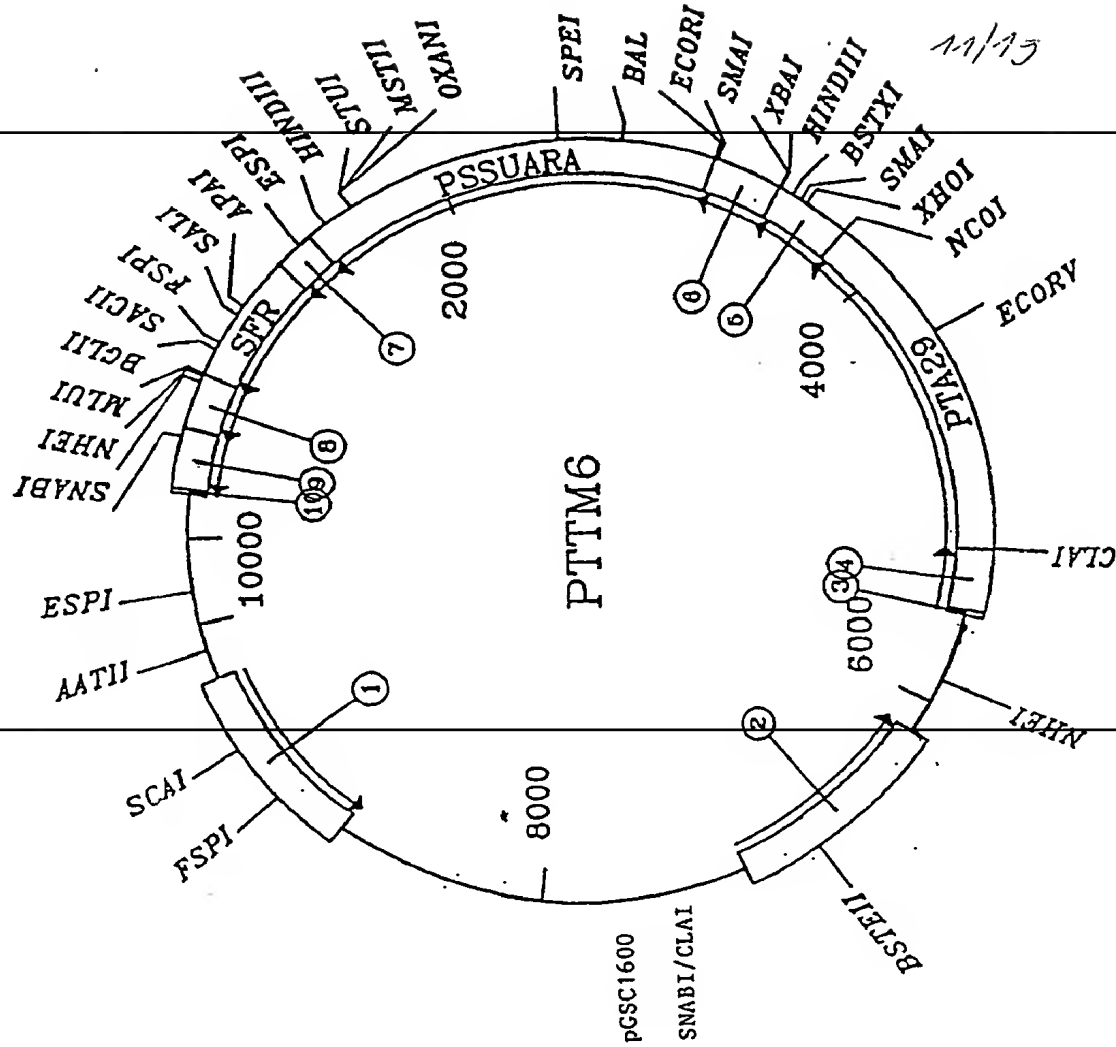


FIG. 1 A

LEGEND

① BETA-LACTAMASE
② SU-SP-AD.TRANSF.
③ LB
④ T-DNA
⑤ RNASE T1
⑥ 3'END NOS
⑦ TP
⑧ 3'END T7
⑨ T-DNA
⑩ RB

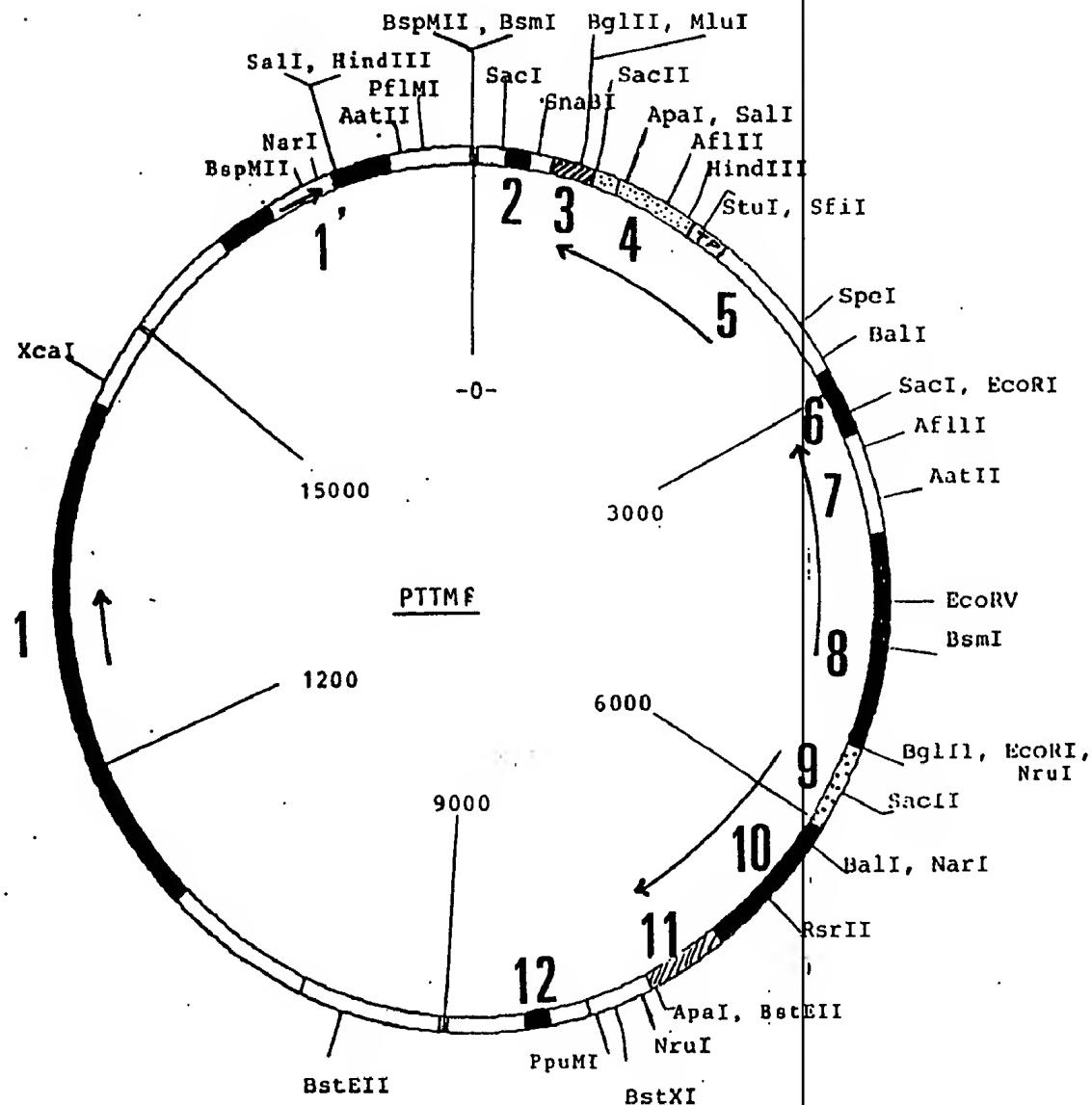


FIGURE 7B
1 : PVS1 ORI
2 : RIGHT BORDER
3 : 3'END T7
4 : SFR
5 : PSSUARA
6 : 3'END NOS
7 : RNASE T1
8 : PTA29
9 : NOS PROMOTOR
10 : NPTII
11 : 3'END OCS
12 : LEFT BORDER
1' : B-LACTAMASE WITH INSERTION

EP 0 344 029 A1

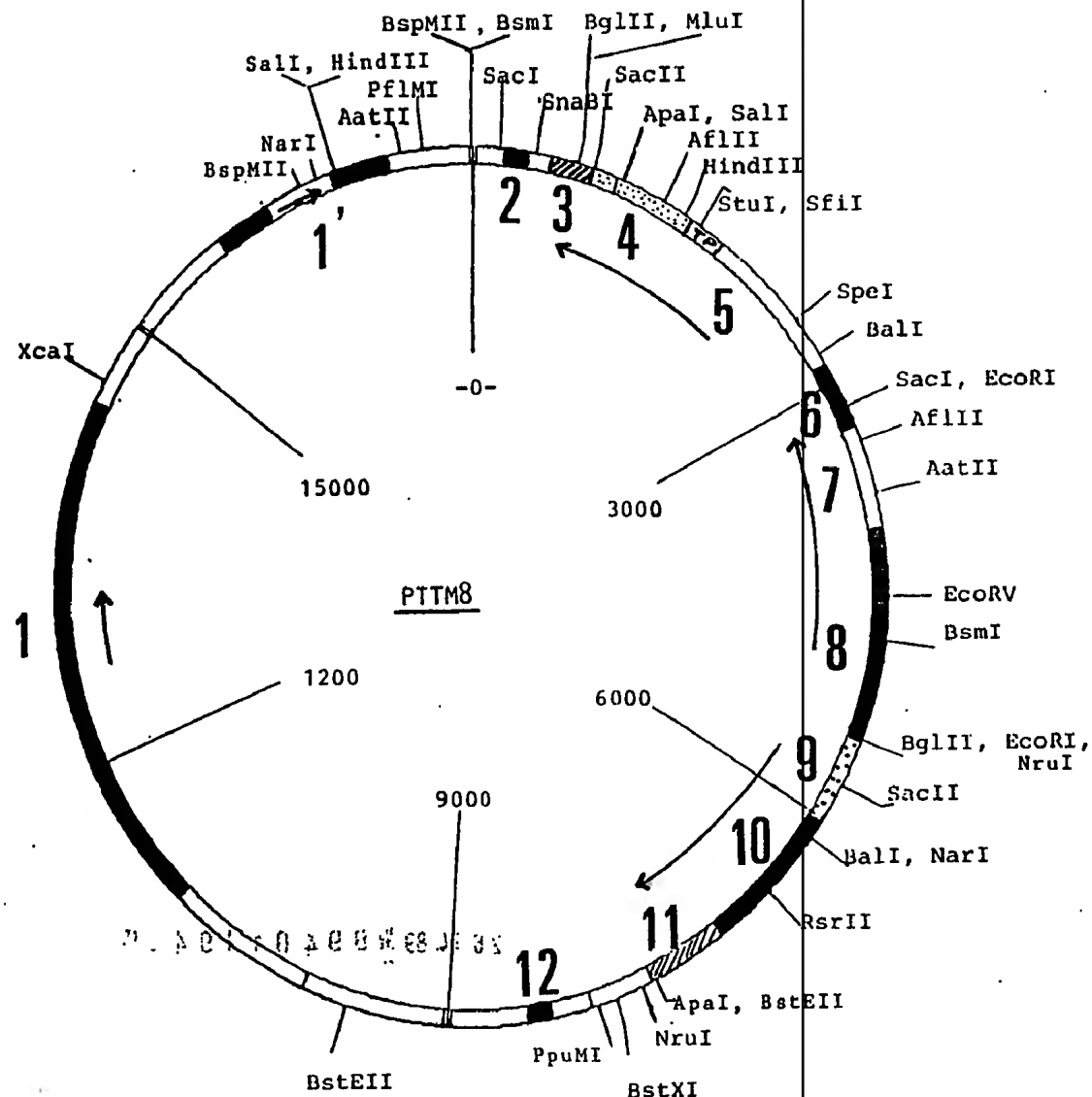


FIGURE 8

1 : PVS1 ORI

2 : RIGHT BORDER

3 : 3'END T7

4 : SFR

5 : PSSUARA

6 : 3'END NOS

7 : BARNASE

8 : PTA29

9 : NOS PROMOTOR

10 : NPTII

11 : 3'END OCS

12 : LEFT BORDER

1' : B-LACTAMASE WITH INSERTION

EP 0 344 029 A1

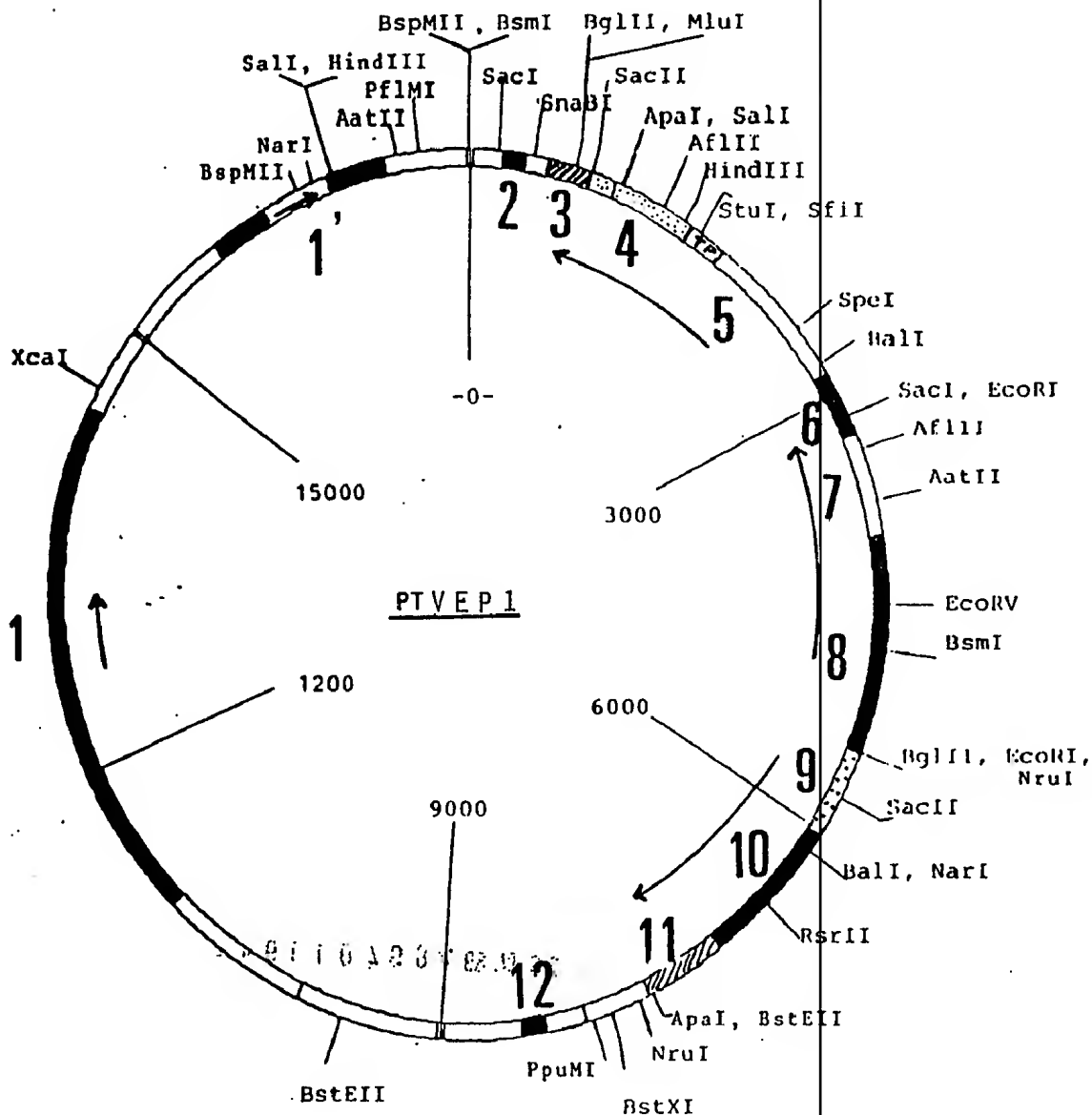


FIGURE 9 A

1	PVS1 ORI
2	RIGHT BORDER
3	3'END T7
4	SFR
5	PSSUARA
6	3'END NOS
7	PAPAIN ZYMOGEN
8	PTA29
9	NOS PROMOTOR
10	NPTII
11	3'END OCS
12	LEFT BORDER
1'	ß-LACTAMASE WITH INSERTION

EP 0 344 029 A1

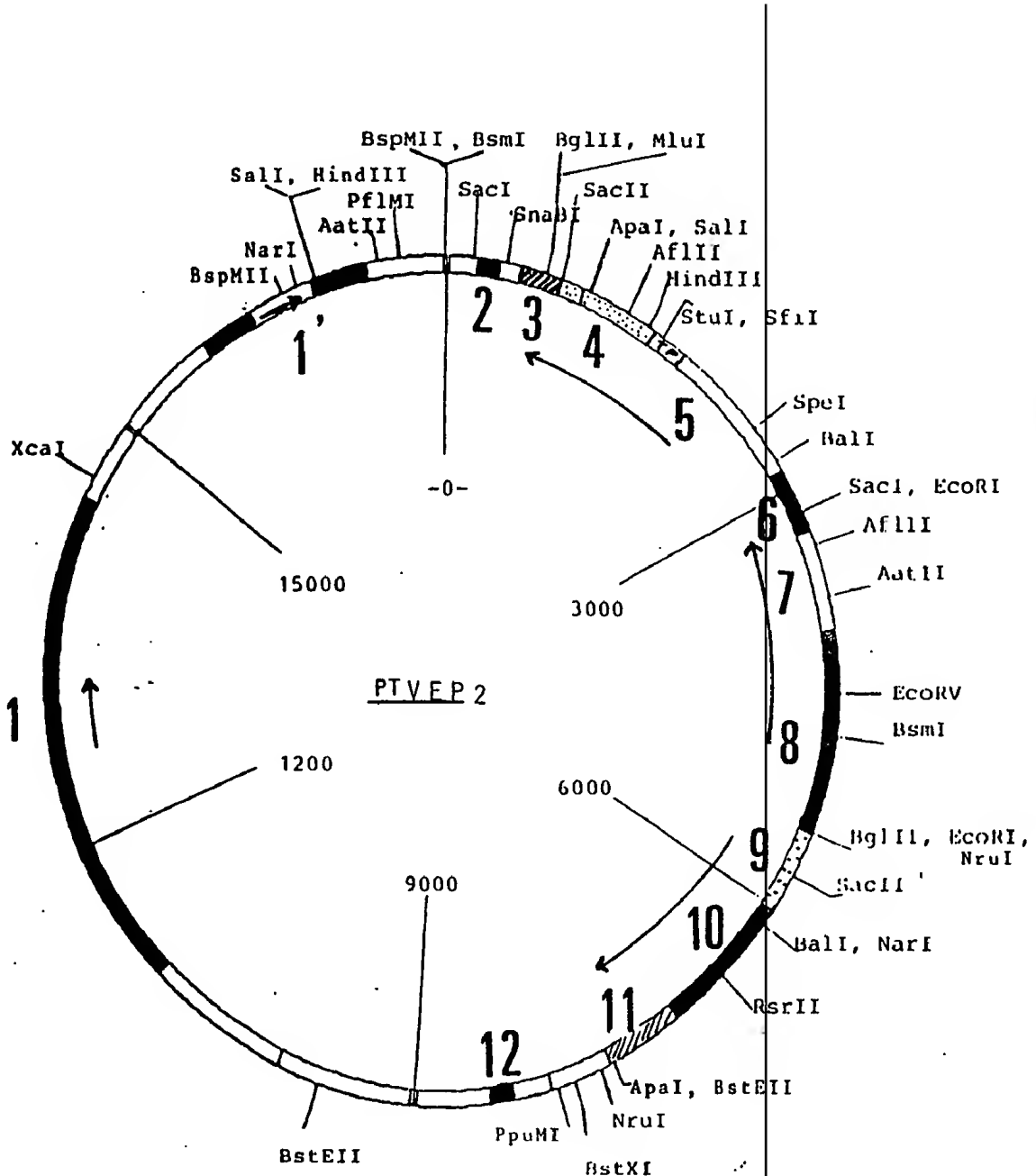


FIGURE 9B
1 : PVS1 ORI
2 : RIGHT BORDER
3 : 3'END T7
4 : SFR
5 : PSSUARA
6 : 3'END NOS
7 : PAPAIN ACT. PROTEIN
8 : PTA29
9 : NOS PROMOTOR
10 : NPTII
11 : 3'END OCS
12 : LEFT BORDER
1' : B-LACTAMASE WITH INSERTION

EP 0 344 029 A1

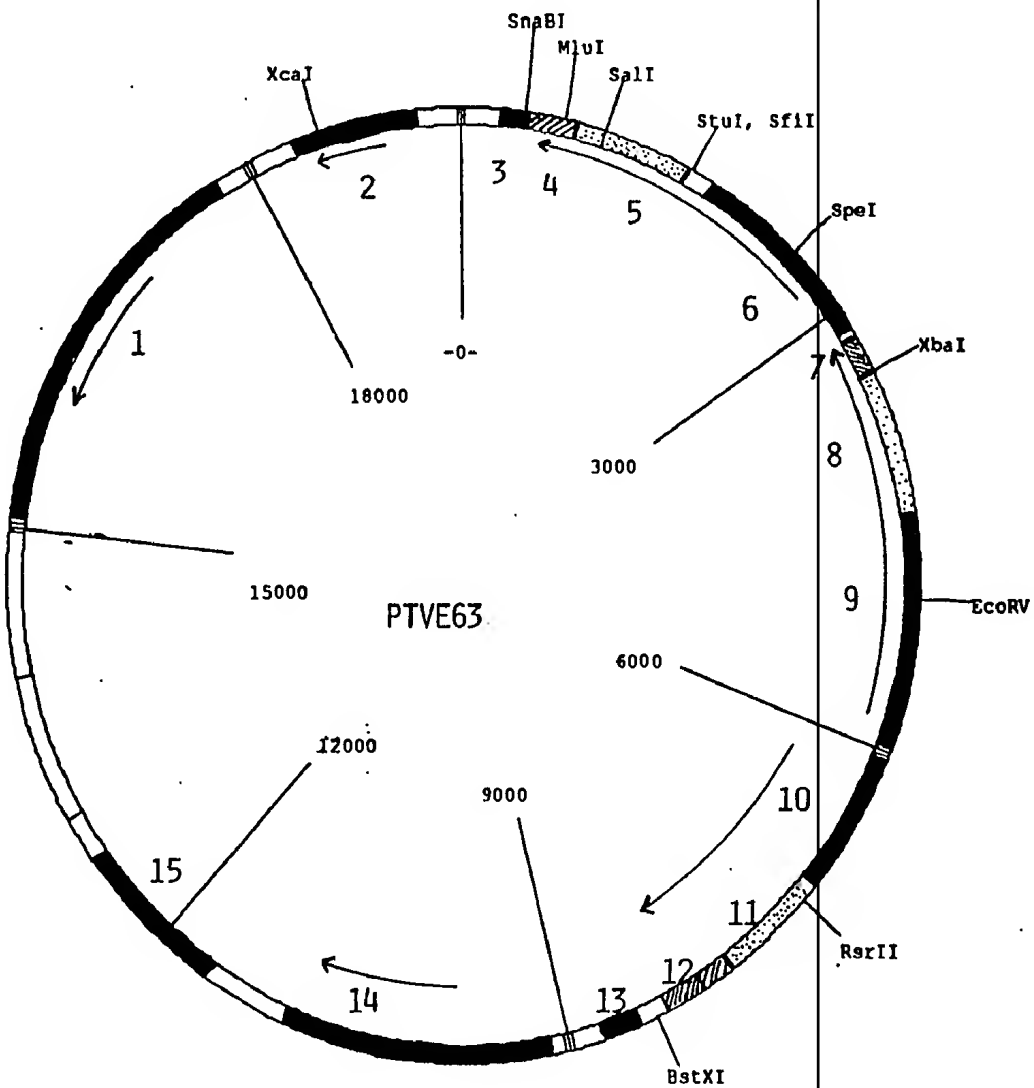


FIGURE 10 A

1 : PVS1 ORI
2 : PBR ORI
3 : RIGHT BORDER
4 : 3' END GENE 7
5 : SFR GENE WITH TRANSIT PEPTIDE
6 : PSSUARA
7 : 3' END NOS
8 : EcoRI
9 : PTA29
10 : NOS PROMOTOR
11 : NPTII
12 : 3' END OCS
13 : LEFT BORDER
14 : METHYLASE RI
15 : SM/SP

EP 0 344 029 A1

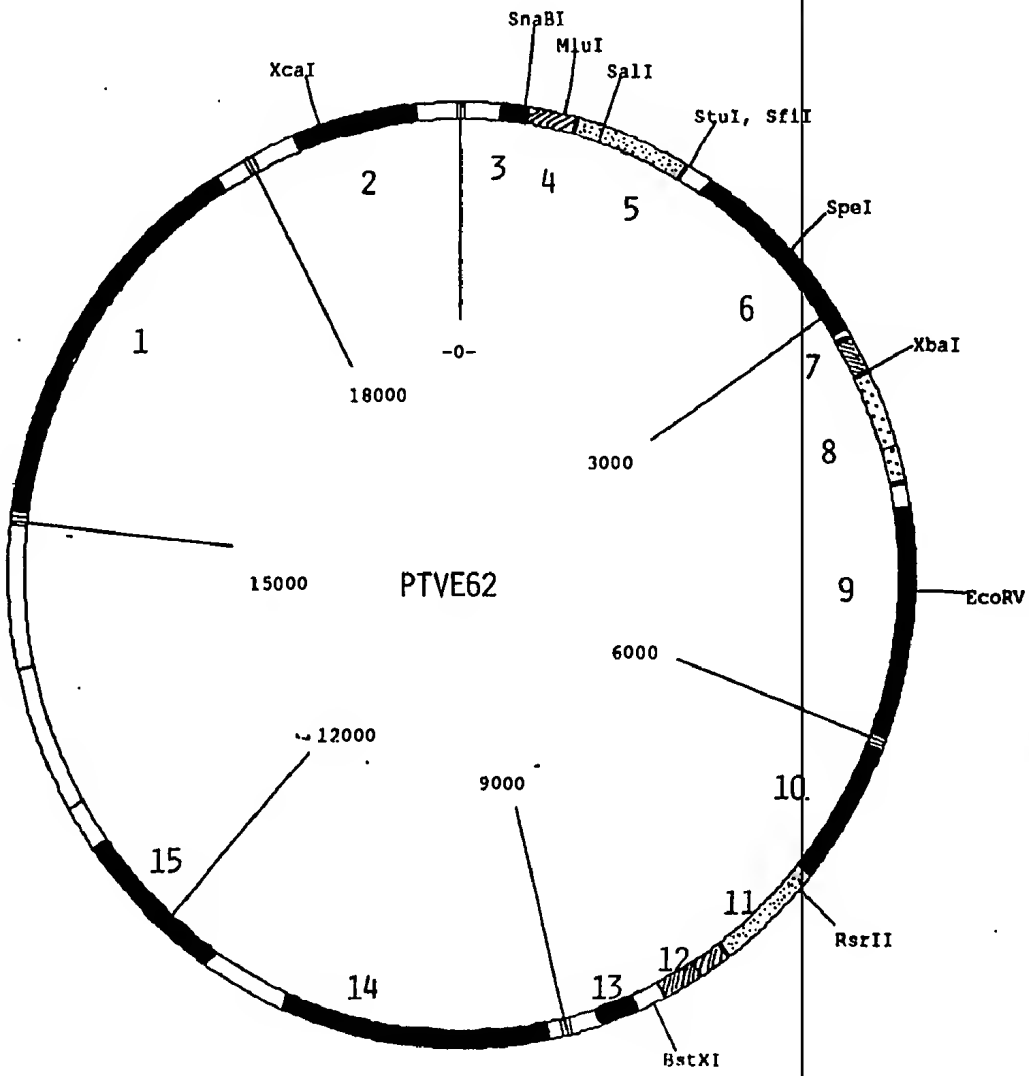


FIGURE 10 B

1 : PVS1 ORI
2 : PBR ORI
3 : RIGHT BORDER
4 : 3' END GENE 7
5 : SFR GENE WITH TRANSIT PEPTIDE
6 : PSSUARA
7 : 3' END NOS
8 : ECORI WITH TRANSIT PEPTIDE
9 : PTA29
10 : NOS PROMOTOR
11 : NPTII
12 : 3' END OCS
13 : LEFT BORDER
14 : METHYLASE RI
15 : SM/SP

EP 0 344 029 A1

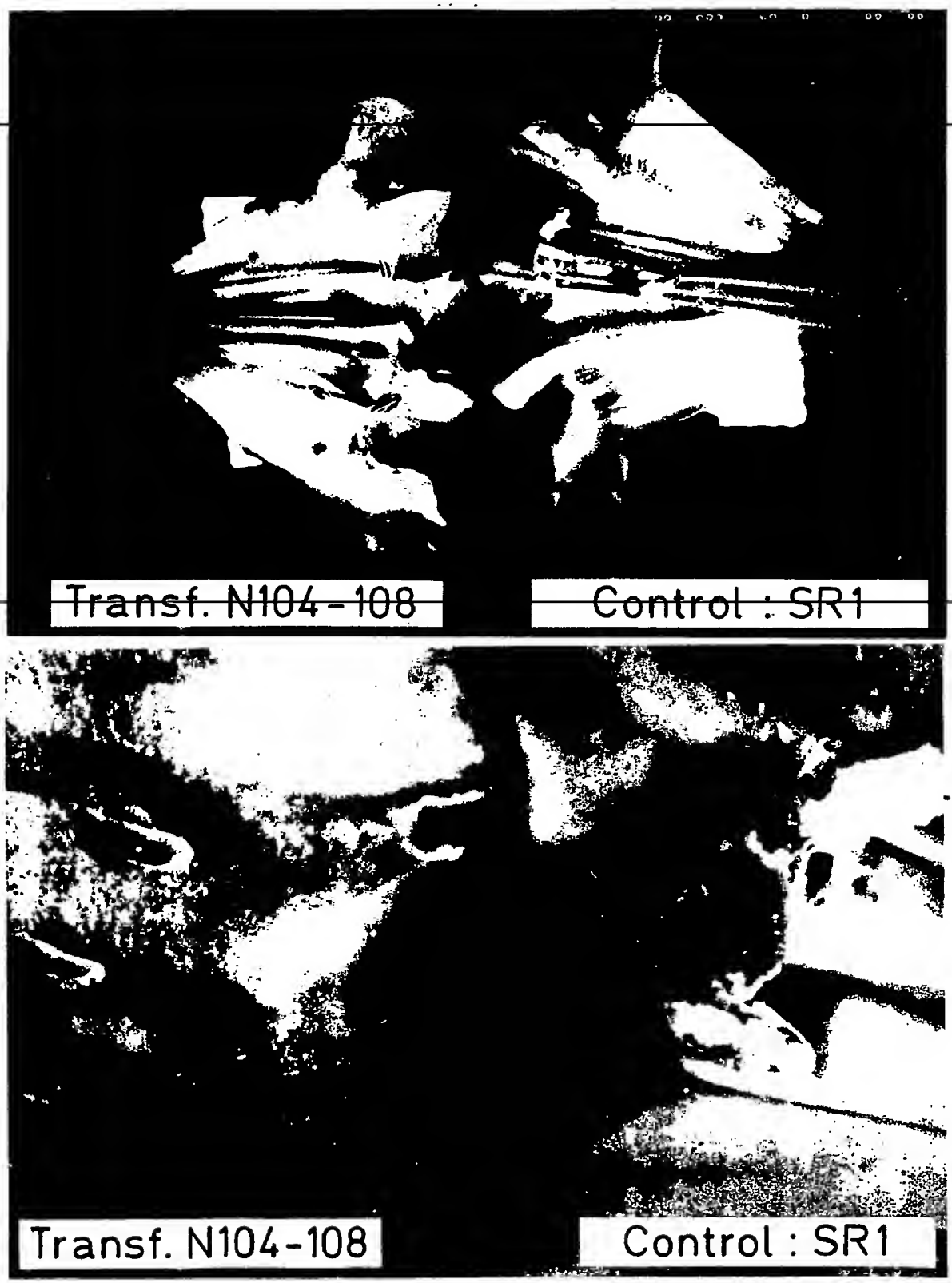
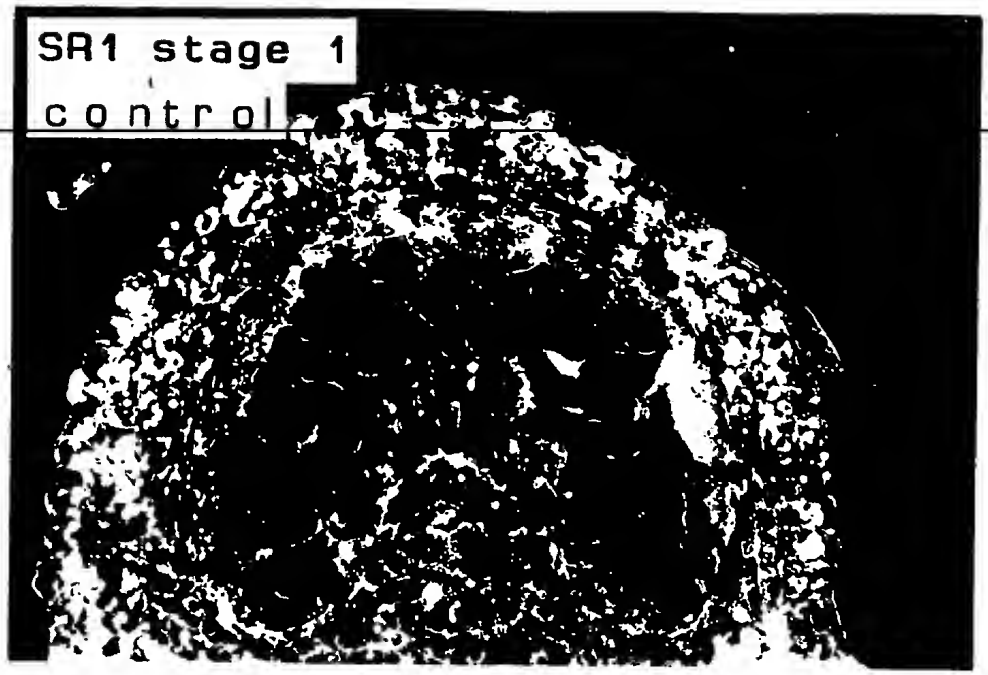
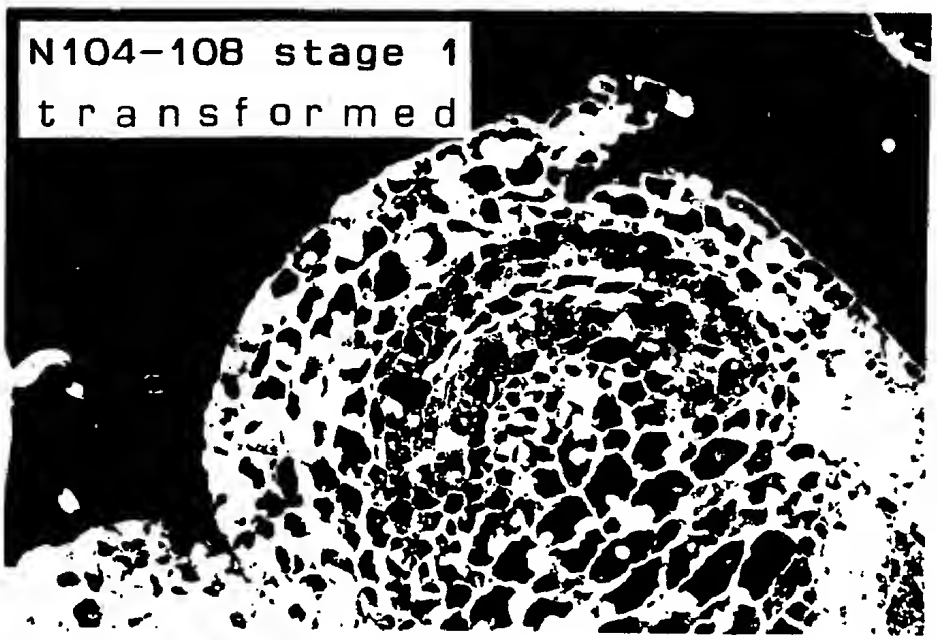


Fig. 11



SR1 STAGE 1, ANTHER CROSS SECTION, THICK TAPETUM AROUND POLLEN SAC, POLLEN STAGE : TETRAD FORM, X250 D.F.



N104-108 STAGE 1, ANTHER CROSS SECTION, THIN LAYER OF TAPETUM, NO POLLEN SAC, NO POLLEN VISIBLE, X250 D.F.

Fig. 12

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 40 1194

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
O,P X	JOURNAL OF CELLULAR BIOCHEMISTRY, Supplement 13D, UCLA SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, ABSTRACTS 18TH ANNUAL MEETINGS, 27th March - 7th April 1989, page 312, abstract no. M349, Alan R. Liss, Inc, New York, US; D. TWELL et al.: "Pollen-specific expression directed by chimaeric genes in transgenic tomato and tobacco plants" * Abstract * ---	1,2,5, 10-13, 20	C 12 N 5/00 C 12 N 15/00 A 01 H 1/00
O,P X	JOURNAL OF CELLULAR BIOCHEMISTRY, Supplement 13D, UCLA SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, ABSTRACTS 18TH ANNUAL MEETINGS, 27th March - 7th April 1989, page 292, abstract no. M257, Alan R. Liss, Inc., New York, US; A.J. VAN TUNEN et al.: "Regulation of chalcone flavanone isomerase (CHI) gene-expression in Petunia hybrida: the use of alternative promoters in corolla, anthers and pollen" * Abstract * ---	1,2,4, 10-13, 20	
O,A	JOURNAL OF CELLULAR BIOCHEMISTRY, Supplement 12C, UCLA SYMPOSIA ON MOLECULAR & CELLULAR BIOLOGY, ABSTRACTS 17TH ANNUAL MEETINGS, 28th February - 10th April 1988, page 137, abstract no. L021, Alan R. Liss, Inc., New York, US; C.S. GASSER et al.: "Analysis of floral specific genes" * Abstract * --- -/-	1-20	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C 12 N
A 01 H

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	16-08-1989	MADOOX A.D.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 40 1194

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
O,A	JOURNAL OF CELLULAR BIOCHEMISTRY, Supplement 12C, UCLA SYMPOSIA ON MOLECULAR & CELLULAR BIOLOGY, ABSTRACTS 17TH ANNUAL MEETINGS, 28th February - 10th April 1988, page 168, abstract no. L137, Alan R. Liss, Inc., New York, US; R. WING et al.: "Molecular characterization of pollen specific cDNA clones from tomato (Lycopersicon esculentum)" * Abstract * ---	1-20	
O,A	BIOLOGICAL ABSTRACTS/RRM, abstract no. BR33:81569; S. McCORMICK et al.: "Identification of genes specifically expressed in reproductive organs of tomato", & PLANT BIOLOGY, vol. 4, Tomato Biotechnology; Symposium, Davis, CA, US, August 20-22, 1986 * Abstract * ---	1-20	
A	EP-A-0 198 288 (ADVANCED GENETIC SCIENCES) * Whole document * ---	1-20	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
P,A	SCIENCE, vol. 240, 10th June 1988, pages 1460-1467; R.B. GOLDBERG: "Plants: novel developmental processes" * Page 1464, left-hand column, paragraph 5 - page 1464, right-hand column, paragraph 1 * ---	1-20	
E	EP-A-0 329 308 (PALADIN HYBRIOS) * Whole document *	1,2,4,6,8,10-14,16,17,19,20	

-/-

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	16-08-1989	MADDOX A.D.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 40 1194

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
O,A	JOURNAL OF CELLULAR BIOCHEMISTRY, Supplement 12C, UCLA SYMPOSIA ON MOLECULAR & CELLULAR BIOLOGY, ABSTRACTS 17TH ANNUAL MEETINGS, 28th February - 10th April 1988, page 212, abstract no. L616, Alan R. Liss, Inc., New York, US; J.I. MEOFORD et al.: "Manipulation of auxin, cytokinin and gus levels using the maize HSP70 promoter" * Abstract * ---	1-20	
O,A	JOURNAL OF CELLULAR BIOCHEMISTRY, Supplement 12C, UCLA SYMPOSIA ON MOLECULAR & CELLULAR BIOLOGY, ABSTRACTS 17TH ANNUAL MEETINGS, 28th February - 10th April 1988, page 138, abstract no. L023, Alan R. Liss, Inc., New York, US; J.P. MASCARENHAS: "Characterization of genes that are expressed in pollen" * Abstract * -----	1-20	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	16-08-1989	MADDOX A.D.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)